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Tree improvement studies in genus *Terminalia* Linn.

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Abstract

Terminalia Linn. (Combretaceae) serves as one of the most important multipurpose tree (MPT) genus due to its vast economic applications in various industries viz tasar silk, pharmaceutical, timber, tannin and leather. Present paper deals with the research progress made till date on the taxonomy, embryology, reproductive biology, cytogenetics, vegetative propagation, conservation and breeding aspects of *Terminalia* species and importance of such achievements in relation to their genetic improvements.

(**Keywords** tree improvement/*Terminalia* Linn /economic botany)

Introduction

Lot of work has already been done on genetic improvement of *Morus* species to enhance the production of mulberry silk and upgradation of the silk quality. On the contrary, very little work has been done on the taxonomy, cytogenetics, conservation and breeding aspects of *Terminalia* species. Recently, tasar culture has also received due attention and hence, research work was initiated on genetic upgradation of food plants of tasar silkworm, *Antheraea mylitta* D. with particular reference to *Pentaptera* section of genus *Terminalia*. Current status and prospects of work done on various biological aspects and studies on improvement of economically important *Terminalia* species are reported in this paper.

(A) Distribution

Genus *Terminalia* Linn. (Combretaceae) comprising of nearly 200 species is distributed throughout the humid and semi-humid tropical and subtropical regions of the world. It is most abundant in Africa and Asia and all the Australian species except *T. microcarpa* appear to be endemic. Nearly 24 species of *Terminalia* have been reported from various states / Union Territories of India viz. Bihar, Jharkhand, Orissa, West Bengal, Madhya Pradesh, Chattisgarh, Uttar Pradesh, Uttaranchal, Maharashtra, Assam, Tamil Nadu, Karnataka, Kerala, Gujarat, Punjab, Rajasthan and Andaman & Nicobar islands.

(B) Economic value

Genus *Terminalia* consists of trees and shrubs of considerable economic importance in tasar culture, timber, pharmaceutical, tannin and leather industries [1-5]. Hence, it has been well recognized as one of the multipurpose trees. A summary of various uses of *Terminalia* species in various industries is given in Table 1.

Table 1– Indian *Terminalia* species as a source of non-wood forest products (NWFP) [1-3,5-12,70].

Sl. No.	Species/taxa	Industry	NWFP	Parts used	Remarks
1.	*T.arjuna* (Syn· *T glabra* & *T berryi)*	Silk (Sericulture)	Tasar	Leaves fed by tasar silkworm, *Antheraea mylitta* D	Considered as back bone of tasar silk industry.
		Pharmaceutical	Indigenous drugs	Bark	Bark considered astringent, diuretic and cardiac stimulant, relieves hypertension, shows presence of calcium and has been used in fever, contusion, fractures, dysentry & ulcers.
				Leaves	Juice of fresh leaves used in earache.

Table 1 Contd .

Table 1 Contd...

		Animal husbandary	Fodder	Leaves	Especially for goats.
		Leather & dyeing	Tannins	Bark and fruits	Tannins form an insoluble compound leather from animal hide
		Soap & Chemical	Arjunalic acid, Saponin, Leucodelphindin & Ellagic acid	Bark/leaves/fruits	Ellagic acid has been considered as insect deterrant
2.	*T tomentosa* (Syn: *T alata*)	Silk (Sericulture)	Tasar	Leaves fed by tasar silkworm, *A mylitta* D.	Considered as back bone of tasar industry.
		Pharmaceutical	Indigenous drugs	Barks	Diuretic, cardiotonic, used in dyspepsia, diarrhoea and also applied on ulcers. Bark yields waxy crystalline substance characteristic of terpenoids
		Animal husbandary	Fodder	Leaves	Especially for goats
		Leather & dyeing	Tannins	Barks & fruits	Bark yields 20% tannins, Bark colour used for dyeing fabrics and cementing of mud walls by tribals.
		Resins & gums	Gum	Trunk	Purgative and adhesive

Table 1 Contd...

Table 1 Contd

		Paper	Pulp	Wood	Wood pulp may be used in mixtures with bamboos and hardwoods
3	*T. paniculata*	Silk (Sericulture)	Tasar	Leaves	Secondary food plant of *A mylitta* D
		Leather & dyeing	Tannins	Fruits & barks	Fruits & bark used for dyeing & tannins
		Papar	Pulp	Wood	Wood pulp may be used in mixtures with bamboos and hardwoods.
		Pharmace-utical	Indigenous drugs	Bark	Bark juice diuretic and cardiotonic.
4.	*T chebula*	Silk	Tasar	Leaves	Secondary food plant of *A mylitta* D.
		Pharmaceutical (Chebullic myrobalans)	Indigenous drugs	Fruits & barks	20% ayurvedic drugs are prepared from this taxa. Astringent, laxative, alterative, dentrifice, diuretic, cardiotonic, cooling and antiseptic Applied for the treatment of ulcers, stomatitis, toothache, bleeding gums, asthma, boils and washing of eyes and colitis
		Leather & dyeing	Tannins	Fruits & Barks	30% tannins obtained from fruits. Ink is manufactured from fruits.

Table 1 Contd...

Table 1 Contd

		Resins & gums	Gums	Trunk	Gum used as adhesive
		Railways	Tannins	Fruits	Fruit extract used for internal treatment of locomotive feed water and as additive in oil drilling compositions.
5	*T bellerica*	Silk	Tasar	Leaves	Secondary food plants of *A mylitta* D
		Pharmaceutical (Belleric myrobalans)	Indigenous	Fruits	25 ayurvedic preparations are made Antibiotic, astringent, tonic, antipyretic and laxative. Given in piles, dropsy, diarrhoea, dyspepsia, headache, leprosy & stomach ailments.
		Leather & dyeing	Tannins	Fruits	Manufacture of leather & ink.
		Soap	Karnel Oil	Fruits	Suitable for wrapping papers.
6.	*T travancorensis*	Pharmaceutical	Indigenous drugs	Bark	Stimulant.
7.	*T. procera*	Leather & dyeing	Tannins	Fruits	Used for dyeing black
8.	*T myriocarpa*	Paper	Pulp	Wood	Wrapping, writing & printing papers are manufactured.

Table 1 Contd...

Table 1 Contd

		Leather & dyeing	Tannins	Bark	Bark yields 18% tannnins
		Pharmaceu-tical	Indigenous drugs	Bark	Cardiac stimulant & diuretic
		Resins & gums	Gums	Bark	Adhesive
9	*T manii*	Leather & dyeing	Tannins	Fruits	Fruits used in low degree tanning.
		Pharmaceu-ticals	Indigenous drugs	Bark	Bark extract diuretic & stomachic
10	*T bialata*	Leather & dyeing	Tannins	Bark	Tanning.
		Pharmaceu-ticals	Indigenous drugs	Bark	Potent cardiac stimulant.
11.	*T coriacea*	Leather & dyeing	Tannins	Fruits	Tanning
		Pharmaceu-ticals	Indigenous drugs	Bark	Potent cardiotonic & heart stimulant
		Silk (Sericulture)	Tasar	Leaves	Primary food plant of *A mylitta* D.
12.	*T crenulata*	Silk (Sericulture)	Tasar	Leaves	Primary food plant of *A mylitta* D
		Leather & dyeing	Tannins	Barks & fruits	Tanning
		Pharmaceu-ticals	Indigenous drugs	Bark	Used for painless delivery by tribals of Chattisgarh.
		Match	Boxes & splints	Wood	Fire ignition
13.	*T. citrina*	Leather & dyeing	Tannins	Fruits	Tannins used as blue dye for colouring fabrics.

Table 1 Contd...

Table 1 Contd...

		Resins & gums	Gums	Trunk	Adhesive
		Pharmaceu-ticals	Indigenous drugs	Bark	Diuretic, cardiotonic
14	*T catappa*	Silk (Sericulture)	Tasar	Leaves	Secondary food plant of *A mylitta* D
		Horticulture	Food	Fruits	Edible fruits (Jangli Badam)
		Oil/Cosmetic	Oil	Fruits	A susbtitute of almond oil, used in cosmetics
		Pharmaceu-tical	Indigenous drugs	Fruits, Bark, Leaves	Rheumatic pains, ulcers, cardiotonic, diuretic, astringent, dysentry, antibiotic and antifungal.
		Resins & gums	Gums	Trunk	Adhesive.
15	*T pallida*	Leather & dyeing & Pharmaceu-tical	Tannins	Fruits	Fruits used as chebulic myrobalans

(C) Taxonomy

The sub-division of the genus *Terminalia* into sections or by some taxonomists into distinct genera, has been founded on the basis of characters of fruit alone, and, although line of demarkation is often very indefinite, no better character has been found so far. Clarke[6] and Brandis [7] have reported 11-13 species indigenous to India and separated them into 3-4 sections viz., *Catappa* (including *Myrobalanus*), *Pentaptera*, *Chuncoa* and an annonymous section allied to *Catappa* on the basis of characteristics of flowers and fruits. On the other hand, Bentham and Mueller[8] separated *Myrobalanus* from *Catappa* and treated only four sections viz., *Catappa*, *Myrobalanus*, *Pentaptera* and *Chuncoa* under genus *Terminalia*. Subsequently, Bentham and Hooker[9] divided *Terminalia* into six sections viz., *Catappa*, *Myrobalanus*, *Pentaptera*, *Chuncoa*, *Bucida* and *Chicharronia*. Blatter [10] catagorized 20 species of *Terminalia* into *Catappa*, *Bialata*, *Pentaptera* and *Chuncoa* sections

with 0, 2, 5 and 3 wings in fruits respectively. Biswas and Kukreti [11], while identifying 20 species with the aid of carpological studies categorized *Terminalia* into five groups viz., I-wingless (9 spp.), II- flatly winged (1 sp.), III- 2 winged (3 spp.), IV- ± 3 wings (1 sp.) and V- 5 winged (6 spp.). However, due to indefinite line of demarkation in fruit characters, Srivastav *et al.*[5] opined that the most appropriate division of *Terminalia* should be into four sections viz., *Catappa, Myrobalanus, Chuncoa* and *Pentaptera.*

T. Citrina, T. bellerica, T. chebula (Section : *Myrobalanus*) and *T. arjuna* and *T tomentosa* (section : *Pentaptera*) exhibit distinct morphotypes distributed in different regions. They may be natural hybrids, different species, subspecies, varieties or ecotypes[12-16] and accordingly they have been regarded as species complexes [17](Table 2). It is worthwhile to mention here that while Hooker has divided *T. tomentosa* into three varieties viz. *typica (alata), crenulata* and *coriacea,* Roth, Wight and Arnott (quoted by Hooker)[14] and Bahadur and Gaur [12] have treated them as distinct species of *Terminalia.* Likewise, *T. arjuna* has also been divided into two varieties viz. *arjuna* and *angustifolia* by Hooker [14]. On the other hand, Thwaites is of the opinion to merge *T. arjuna* and *T. tomentosa* into one species [12,18]. Four explorations conducted during 1987, 1989, 1990 and 1993 indicate existence of 12 taxa/or morphotypes in *T. arjuna and T. tomentosa* [19]. Such distinctions have been made on the basis of leaf, stem and fruit characteristics encountered within these two species. Apart from morphogenetic diversity, physiogenetic diversity also occurs in *T. arjuna* and *T. tomentosa* [20]. Intra-specific diversity encountered in *T. citrina, T. chebula, T. bellerica, T. arjuna* and *T. tomentosa* species complexes revealed overall existence of two, six, three, four and eight taxa respectively within these species complexes (Table 2).

Table 2– Intraspecific diversity in *Myrobalanus* and *Pentaptera* sections of *Terminalia*[12-15,17]

Sl. No.	Section/Taxa	Distinguishing features.
A	Section: *Myrobalanus*	Fruit globular or ovoid, terete or slightly compressed or surrounded by a prominant acute angle but not distinctly winged.
1.	*T citrina* Roxb.	Leaves oblong-elliptic, acuminate, narrowed into petioles, drupe 5- angled
	var 1 *typica*	Petioles less than $^1/_2$ in., fruit nearly 2 in. oblong-lanceolate, obscurely 5-angular, found in Assam and West Bengal.
	var. 2. *malayana* Kurz.	Petioles more than $^1/_2$ in., fruits less than 2 in., found in Nicobar.

Table 2 Contd...

Table 2 Contd.

2	*T bellerica* Roxb	Leaves broadly elliptic, leathery with raised nerves and reticulations, attenuated at branch apex, fruits ellipsoid-globular, hairy, obscurely 5-angled
	var. 1. *typica*	No glands at the apex of the petiole
	var. 3 *laurinoides* Miq.	Leaves obovate or obovate-elliptic, shortly acuminate, much thinner than in the typical *bellerica*
	var 2 b*ellerica* Roxb	Two glands at the apex of petiole
3.	*T chebula* Retz.	Leaves ovate or elliptic. scattered, acute, rounded at base, ellipsoid fruit glabrous and 5-ribbed
	var. 1. *typica*	Adult leaves nearly glabrous beneath, young ovary shaggy outside, calyx teeth glabrous outside, common in Deccan
	var. 2	Adult leaves glabrous beneath, young ovary glabrous, calyx teeth pubescent, common from Kumaon to Bengal and Chotanagpur
	var 3.	Adult leaves very shaggy beneath, fruit only $^3/_4$ in., fround on the summit of Parasnath Behar alt 4000 ft. ASL
	var 4 *tomentella* Kurz	Young leaves coppery-pubescent beneath, adult leaves pubescent or glabrescent beneath, young ovary glabrous, fruit ovoid, hardly 1 inch.
	var 5 *gangetica* Roxb.	Adult leaves with brown red silky hairs on both surfaces, found on the bank of Ganges
	var. 6. *parviflora* Thwaites	Calyx teeth glabrous.
B	Section· *Pentaptera*	Fruits ovoid with 4-5 or more than 5 acute sub-equal wings Spikes usually panicled.
1	*T arjuna* Bedd.	Fruits 1-2 in., bark smooth, grey, flaking off in large thin layers, wings not broad, their striations curving upwards, leaves glabrous.
	(I) *T berryi* W & A syn. *T arjuna* var. *angustifolia*	Leaves narrow, elongate-oblong, suddently narrowed into petiote, branches drooping. L/B >4
	(II) *T glabra* W. & A. syn. *T. arjuna* var. *arjuna*	Leaves often cordate, obtuse or acute with horizontal branches. L/B <4.
	(III) Small leaf form	Leaves elliptic-lanceolate L/B=3.0-3.5
	(IV) Putative hybrids closer to *T arjuna*	Bark grey, slightly rough, leaves broader than those of *T glabra,* not perfectly glabrous, veins not buldging.

Table 2 Contd. .

Table 2 Contd .

2	*T tomentosa* W & A.	Bark rough, grey-black, not flaking in thin layers, wings very broad, striations of wings horizontal upto edge, leaves tomentose rarely glabrous
	(I) *T alata*	Leaves obcordate or oblong, suddently narrowed into the petioles, adult hairy beneath, bark dark grey-black, young ovary villous, inflorescence hairy, fruits glabrous, bark exfoliating in irregular flakes
	(a) *T alata* var *alata* Heyne ex Roth syn *T tomentosa* var *alata*	Leaves elliptic, oblong or ovate-oblong, 15x8 cm , thinly wooly beneath, found in Central India L/B= 2.0-2.5
	(b) *T alata* var. nepalensis (Haines) Fernandes syn. *T tomentosa* var. *nepalensis*	Leaves oblong, elliptic or lanceolate, 13-30 x 4-10 cm , strongly tomentose beneath, distributed in northern India L/B>3.
	(II) *T crenulata* Heyne ex Roth. syn. *T tomentosa* var. *crenulata*	Leaves narrowed into the petiole, obovate to epllıptic, adult nearly glabrous beneath, young ovary and fruits glabrous, common in Western Ghats Bark dark grey, exfoliating in rectangular flakes.
	(a) Big leaf forms	Obovate leaves
	(b) Small leaf forms	Oblong leaves
	(c) Kahvi	Elliptic leaves
	(III) *T coriacea* (Roxb) W & A. syn. *T tomentosa* var *coriacea*	Bark dark coloured like crocodile skin, leaves like *T alata* but beneath with a close hard fulvous. tomentum rather than villous, fruits pubescent between wings Common along Eastern Ghats, bark exfoliating in squarish flakes
	(a) Big leaf forms	Obovate leaves.
	(b) Small leaf forms	Oblong leaves.
	(IV) Putative hybrids closer to *T tomentosa*	Bark greyish black, rough and slightly cracked, leaves bigger than those of *T glabra,* glabrous but not perfectly smooth, veins slightly buldging.

(D) Embryology

Ovule is bitegmic and crassinucellate with "Polygonum" type of embryosac development in *Terminalia* species. While "Ongrad" type of embryo development has been observed in *T. catappa,* "Solanad" type of development has been noticed in *T. bellerica (*section : *Myrobalanus*). In *T catappa* cleavage polyembryony has been noticed[21].

In *T. arjuna* and *T. tomentosa* (section : *Pentaptera*), the occurrence of monocotyledonous, tricotyledonous, tetracotyledonous, twin and triplet seedlings was recorded[22,23,25]. Their frequency seems to be 0.199, 0.478, 0.022, 0.206 and 0.006% in *T. arjuna.* In *T. tomentosa* only twins (0.16%) have been recorded. In *T. bellerica,* 2-4% seeds exhibited twin seedlings. In some seedlings of this species three plumules were also noticed [24].The twins and triplet seedlings seem to have arisen more due to false polyembryony than due to cleavage polyembryony and twins might have produced tetracotyledonous and tricotyledonous seedlings due to their fusion followed by abortion of a embryo and/or a cotyledon in nature. The occurrence of such anomalies in *Terminalia* may be genetic as the progenies of 8 plus trees exhibited no seedling anomalies while 14 plus trees had anomalous seedlings [23].

(E) Reproductive biology

Studies on reproductive biology of *Terminalia* species were concentrated on their flowering behaviour, floral biology and pollination mechanisms in order to chalk out effective breeding programmes.

(i) Flowering behaviour

Juvenile flowering was observed in the seedlings of *T. arjuna* (0.0066%) and *T. tomentosa* (0.0006%) during mass nursery and progeny testing of 24 plus trees at the age of 3-4 months. The sexually early maturing plants of *T. arjuna* (0.0081) second year onwards and early and late flowering plants of *T. arjuna* (0.03 and 0.05%) and *T. tomentosa* (0.06 and 0.03%) were also isolated in raised plantation and their flowering period, floral biology and cytology were investigated which opens avenues for evolution of seedling flowering and early maturing strains [26].

(ii) Floral biology

Studies on anthesis and floral biology were conducted in four species of *Terminalia* which revealed that they differed significantly from each other barring very few characters, where very less differences were discernible. The smallest buds

(1.2 x 1.3 mm) and sepals (1.7 x 1.7 mm) were found in *T. chebula* and biggest in *T. arjuna* (2.5 x 1.67 and 3.07 x 2.87 mm, respectively). Highest number of flowers/inflorescences were observed in *T. tomentosa* (560) and lowest in *T. chebula* (340). The length of stamens, styles and fruits were highest in *T. arjuna* and lowest in *T. paniculata*. Floral biology of *T. arjuna* is much closer to *T. tomentosa* than to *T. chebula* while *T. paniculata* differed from all the three species. Such closeness might have resulted in production of natural hybrids between *T. arjuna* and *T. tomentosa* in distant past. Anthesis, anther dehiscence and stigmatic receptivity have been found to be closely associated with temperature, relative humidity, cloudy weather and rain in all the four species [27].

(iii) Pollination mechanism

Studies on the pollination mechanism of above four species of *Terminalia* viz. *T. arjuna, T. tomentosa, T. chebula* and *T. paniculata* revealed that pollination is entomophillous. A total of 21 out of 22 insect pollinators collected were identified which belonged to Lepidoptera, Hymenoptera, Hemiptera, Coleoptera and Diptera orders. Maximum number of pollinators belonged to Lepidoptera (6) , Diptera (6) and Hymenoptera (6). Among these, both solitary bees like *Allodope marginata* and domesticated bees like *Apis florea* and *A. indica* play very important role in pollination. The studies reveal that the domesticated bees may be utilized for enhancing pollination and evolution of interspecific hybrids [28]. In *T. paniculata,* of the various insects visiting flower heads, butterflies were most conspicuous and 11 species belonging to 4 families and 10 genera were identified. The family Pieridae (5 spp.) was most abundant followed by Danaidae (3 spp.), Nymphalidae (2 spp.) and Papilionidae (1 sp.) [29].

(F) Genetics

(i) Genetic Variability

Studies on genetic variability were carried out for fruits, seedlings, leaves and leaf yield characters as a fundamental basis for selection.

(a) Variability in fruits

The variability studies in fruits of 22 spontaneous hybrid plus trees of *T. arjuna* and *T. tomentosa* marked in Chattisgarh and Orissa and their 2 controls from Jharkhand (Table 3) revealed that high heritability values for fruit weight, breadth of wings and germination percentage were associated with high genetic advance

indicating additive gene effects for these characters, whereas low/moderate genetic advance against high heritability for length of fruits indicates intra or interallelic interactions for this character [30]. Variability studies of other 10 superior trees of *T tomentosa* revealed that maximum germination (32.22%) was exhibited by treatment number 5 while minimum germination was noticed in treatment number 3(13.33%) [31].

Table 3– Variability and genetic parameters of fruit characters in *T arjuna* and T. tomentosa complexes[30].

Parameters	Fruit weight(g)	Length of fruits (cm)	Breadth of wings of fruits (cm)	Germination percentage
1 Range	5 4-1 47	5 71-2 99	1 77-0 50	90 00-21 00
2 Mean	3 313	4 473	1 094	65 681
3 SE (Mean)	0 050	0 058	0 056	4 523
4 C D at 5%	0 143	0 166	0 161	12 876
5 Phenotypic variance	1 3587	0 5164	0 140	367 2878
6 Genotypic variance	1 3511	0 5062	0 1304	305 9135
7 Error variance	0 0076	0 0102	0 0096	61 3743
8 Phenotypic coefficient of variance (PCV)	35 18	16 06	34 20	29 18
9 Genotypic coefficient of variance (GCV)	35 08	15 91	33 01	26 63
10 Genetic advance in percentage of means	72 07	32 44	65 44	55 06
11 Heritability (H%)	99 44	98 02	93.17	83 29

(b) Variability in half-sib seedlings

Variability studies in half-sib seedlings belonging to aforesaid 24 plus trees revealed high heritability, high genetic advance and high genotypic coefficient of variation for seedling height which indicates additive gene action for this character whereas low GCV, low genetic advance and high heritability were noticed for leaf length, leaf breadth and L/B ratio suggesting thereby intra- and interalletic interaction for these leaf characters[32]. Variability studies of 10 superior trees of *T. tomentosa*

revealed that seedling height ranged from 25.07 cm in treatment 8 to 39.24 cm in treatment number 2[31].

(c) Variability in leaves

Genotypic variability of 9 foliar characters studied in 39 plus trees of *T. tomentosa, T. arjuna* and natural hybrids revealed maximum variation in leaf weight followed by L x B and stomatal frequency. High GCV and PCV were reported for leaf weight, leaf area, L x B, breadth and length of leaf and stomatal frequency in descending order. The high heritability was associated with high genetic advance for L x B and leaf area which confirms additive gene action for these two characters whereas low / moderate genetic advance against high heritability for other seven foliar characters viz. length, breadth, weight and L/B ratio of leaves and length, breadth and frequency of stomata indicates intra- and interallelic interactions for these characters[33].

(d) Variability in leaf yield

The maximum range of variation was recorded in leaf yield/plant followed by breadth of leave. Whereas GCV was highest in leaf yield followed by No. of leaves/branch, highest PCV in leaf yield/plant was followed by breadth of leaf. The highest heritability was noticed for leaf yield/plant followed by No. of leaves/branch. The highest genetic advance was, however, observed for plant height followed by No. of leaves/branch. Hence, leaf yield/plant, No. of leaves/branch and height of the plants should be considered as effective parameters for selection in *T arjuna* for increasing productivity of tasar silk/unit area [34].

(ii) Correlation studies

Studies on inter-relationship of various characters in breeding programmes are helpful for making indirect selection. For such purpose formulae suggested by Miller *et al.* [35] were adopted for calculating genotypic and phenotypic correlation coefficients.

(a) Correlation between fruit characters

The fruit length was found to be significantly and positively correlated with fruit weight and breadth of wings as compared to non-significant positive correlation between fruit weight and breadth of wings and negative correlation of germination percentage with other fruit characters. Such studies were performed in 24 populations of both the species and their hybrids[30].

(b) Correlation between foliar characters

The foliar characters viz. area, length, breadth, L x B, weight and stomatal length had significant positive correlation with each other while stomatal frequency had significant negative correlation with these foliar characters. These studies were performed in 39 genotypes of *T. arjuna, T. tomentosa* and their hybrids from Jharkhand[36].

(c) Correlation between leaf yield and yield contributing characters

Correlation studies carried out in the progenies of 24 genotypes of *T. arjuna* Bedd., *T. tomentosa* W. & A. and their natural hybrids revealed positive and highly significant correlation of leaf yield with number of branches and leaves/plant and highly significant negative correlation with length and breadth of leaves [37](Table 4).

Table 4 : Phenotypic and genotypic (within parenthesis) correlation co-efficient between various plants characters in *T. arjuna* and *T tomentosa* complexes [37].

Sl No	Character	1	2	3	4	5	6
1	Plant height (cm)	1 00	-0 237	-0 026	-0 123	-0 091	-0 055
			(-0 231)*|	(0 130)|	-0 200	(0 298)*|	(0 053)|
2	No. of branches/ plant		1 00	0 395**	-0 234	-0 428**	0 539***
				(0 782)***	(-0.612)***	(-0.783)***	(0 791)***
3	No. of leaves/plant			1 00	-0 441**	-0 604***	0 712***
					(-0 945)***	(-0 888)***	(0 979)***
4	Length of leaf (cm)				1 00	0 741***	-0 446**
						(0 877)***	(-1.013)***
5	Breadth of leaf (cm)					1 00	-0.535***
							(-0 834)***
6	Leaf yield/plant						1 00

*,**,*** - Significant at 5%, 1% and 0 1% levels respectively.| - imaginary value.

(d) Correlation between photosynthetic rate and other physiological parameters

Net photosynthetic rate (P_N), chlorophyll, protein and starch content of leaves of *Terminalia arjuna* were estimated monthly from Sept. 1990 to Aug. 1991. A positive correlation was observed between net photosynthetic rate (P_N), chlorophyll, protein and starch content of leaves which may facilitate growth and development of tasar silkworm to increase tasar silk production [38].

(iii) Coheritability studies

Coheritability has been considered as a more reliable genetic parameter than correlation for enhancing the efficiency of plant selection since it permits the study of changes in pairs of characters due to environmental effects. Coheritability has been calculated by formula suggested by Nei [39].

(a) Coheritability for fruit characters

The coheritability of fruit weight with length of fruits and breadth of wings were higher than the heritability of fruit weight alone which indicates pleiotropism/linkage between these three characters [30] (Table 5).

Table 5– Estimates of Coheritability, heritability (without parenthesis), phenotypic correlation coefficients (within single parenthesis) and genotypic correlation coefficients (within double parenthesis) for fruit characters in *T arjuna* and *T tomentosa* complexes [30].

	Characters	Fruit weight	Fruit length	Breath of wings of fruits	Germination percentage	Heritability
1	Fruit weight	1 00	0 9948	1.8048	0.7305	0 9944
			(0.8333)**	(0 1241)	(-0.0098)	
			((0 8397))**	((0 2326))	((-0 0078))	
2.	Length of fruits		1 00	0 9314	0 8218	0 9802
				(0 3193)*	(-0 1047)	
				((0.3112))*	((-0.0952))	
3.	Breath of wings of fruits			100	-0.4529	0 9317
					(-0 0426)	
					((-0 0219))	
4.	Germination percentage				1 00	0 8329

(b) Coheritability for foliar characters

The coheritability of leaf area, leaf weight, leaf length, L/B ratio, LxB and stomatal frequency with each other was found to be higher than their corresponding heritability alone which is due to linkage/pleiotropism between these six foliar characters [40].

(c) Coheritability for leaf yield

The coheritability of number of branches and leaves/plant, length of leaf, breadth of leaf and leaf yield/plant have been found to be higher than their corresponding heritability alone which is due to pleiotropism/linkage between these characters[37] (Table 6).

Table 6– Estimates of coheritability of dirfferent pairs of plant characters and their heritability in 24 genotypes in *T. arjuna* and *T tomentosa* complexes[37].

Sl. No.	Characters	Coheritability					Heritability
		2	3	4	5	6	
1	Plant height	0 236	-0.883	0.182	-0.572	-0 173	-0.050
2.	No.of branches/plant		1.210	1.027	1 119	0.908	0 609
3	No.of leaves/ plant			0.811	0 903	0.854	0 613
4.	Length of leaf				0.467	0.905	0.253
5.	Breadth of leaf					0.907	0.615
6	Leaf yield(gm/plant)						0 628

(iv) Path analysis

Path analysis provides more clear picture than correlation studies alone. Hence, path coefficient analysis was carried out at the phenotypic and genotypic levels following the method of Dewey and Lu[41].

(a) Path analysis in *T. arjuna*

Path coefficient analysis revealed that plant height and No. of leaves/branch have direct effect on leaf yield and hence these two attributes should be given greater importance in conjunction with breadth of leaf while formulating selection indices for *T. arjuna* for tasar culture[42].

(b) Path analysis in 24 genotypes of *Terminalia arjuna, T. tomentosa* and their natural hybrids

The highest positive phenotypic direct effect on leaf yield was exhibited by No. of leaves/plant followed by No. of branches/plant which resulted into their highest correlation with leaf yield. On the contrary, the high phenotypic negative correlation of leaf length with leaf yield was partly due to its direct and partly due to indirect effect via plant height, No. of branches/plant and No. of leaves/plant whereas the highly negative phenotypic correlation of breadth of leaf with leaf yield was solely due to its indirect effect via plant height, No. of branches and leaves/plant and leaf length. Such cumulative studies indicate that No. of branches and leave/plant should be considered as effective parameters of selection for higher leaf yield [37](Table 7).

Table 7– Phenotypic and genotypic (within parenthesis) direct and indirect effects of five characters on leaf yield in *T arjuna* and *T tomentosa* complexes [37].

Sl No	Characters	1	2	3	4	5	Correlation coefficients
1	Plant height	0 0122	-0 0738	-0 0143	0 0213	-0 0054	-0.0550
		(4.0177)	(-0.6760)	(-0 7240)	(-0 4236)	(-2 1411)	(0.0530)
2	No.of branches/plant	-0.0041	0.3112	0 2170	0.0405	-0.0256	0.5390***
		(1.2897)	(2.1059)	(-4 3549)	(-1 2962)	(5 6258)	(0.7910)***
3	No.of leaves/plant	-0.0004	0 1229	0 5493	0 0764	-0.0362	0.7120***
		(0.5223)	(1 6468)	(-5.5689)	(-2.0014)	(6.3802)	(0.9790)***
4.	Length of leaf	-0.0021	-0.0728	-0 2422	-0.1732	0.0444	-0.4460**
		(-0 8035)	(-1.2888)	(5.2626)	(2 1179)	(-6.3012)	(-1.0130)***
5	Breadth of leaf	-0.0016	-0.1332	-0.3318	-0.1283	0 0599	-0.5350***
		(1.1973)	(-1.6489)	(4.9452)	(1.8574)	(-7.1949)	(-0.8340)***

(v) Clustering of genotypes

In order to have an extensive breeding programme in *Terminalia* species for hybridization for high yields, response to inputs and also for increase in productivity and improvement in quality of tasar silk, informations on genetic diversity were accumulated.

(a) Fruit diversity

The 22 superior trees (genotypes) exhibiting mixed morphological characters and two typical morphotypes of *T. arjuna* and *T. tomentosa* fell into ten clusters.The fruit weight, fruit length, breadth of wing and germination percentage were found to contribute 32.97, 32.67, 16.30 and 18.11 percent, respectively towards divergence (Tables 8,9). The intercluster D^2 values ranged from 44.61 to 2367.06 suggesting very wide diversity between the populations. The clustering revealed lack of correlation between geographical distribution and genetic divergence. The cluster means for four fruit characters have exhibited very wide variation (Table 10). The canonical analysis has also confirmed the fruit diversity as measured by D^2 statistics [43].

Table 8– Source of origin and clustering pattern of 24 plus trees of *T arjuna* and *T tomentosa* complexes[43,44].

	FRUIT CHARACTERS			(B) SEEDLING CHARACTERS			
Cluster	No. of plus trees	Plus trees	Source	Cluster	No of plus trees	Plus trees	Source
I	2	B5	Sundargarh, Orissa	I	6	N1	Raipur-Jagadalpur Road,Chattisgarh
		N6	Umerkote, Orissa			N4	--do--
						O2	Sundargarh, Orissa
						S2	--do--
						S1	--do--
						B4	--do--
II	4	B3	Sundargarh	II	6	N2	Raipur-Jagadalpur Road
		N1	Raipur-Jagadalpur Road, Chattisgarh			DS2	Dhamtari, Sorgaon
		N3	--do--			DS4	--do--

Table 8 Contd. .

		DS4	Dhamtari, Sorgaon			D	Dhamtari,
						N6	Umerkote,
						DS1	Dhamtari Sorgaon
III	3	B6	Sundargarh	III	4	B5	Sundargarh,Orissa
		D	Dhamtari, Chattisgarh			B6	--do--
		C1	CTR&TI,Ranchi			B1	--do--
						S3	--do--
IV	2	B4	Sundargarh	IV	4	N5	Umarkote,Orissa
		N4	Raipur-Jagadalpur Road			DS3	Dhamtari,Sorgaon
						B2	Sundargarh,Orissa
						N3	Jagadalpur-Raipur Road.
V	4	B2	Sundargarh	V	1	O1	Sundargarh,Orissa
		O2	--do--				
		S3	--do--				
		C2	CTR&TI,Ranchi				
VI	2	S2	Sundargarh	VI	1	B3	--do--
		S1	Sundargarh				
VII	2	B1	Sundargarh	VII	1	C2	CTR&TI,Ranchi
		DS1	Dhamtari, Sorgaon				
VIII	2	N2	Raipur-Jagadalpur Road	VIII	1	C2	--do--
		DS2	Dhamtari, Sorgaon				
IX	2	N5	Umerkote				
		DS3	Dhamtari, Sorgaon				
X	1	O1	Sundargarh				

Table 9– Anova showing F values and independent contribution towards divergence in *T arjuna* and *T tomentosa* complexes [43-45]

Sl.No.	Characters	Mean Sum of squares	F values	Ranked I (No of times)	Contribution in divergence (%)
(A) Fruit Characters (Chattisgarh, Jharkhand & Orissa · 24 genotypes)					
1.	Weight of fruits	4 06	539.11*	91	32 97
2	Length of fruits	1 43	150 31*	90	32 67
3	Breadth of wings	0 40	41.80*	45	16.30
4.	Germination (%)	979 12	15 95*	50	18 11
(B) Seedling characters (Chattisgarh, Jharkhand & Orissa . 24 genotypes)					
1.	Leaf length	3 943	6 59**	7	2 54
2.	Leaf width	0 920	8 65**	10	3 62
3	L/B	1.268	20.26**	16	5 80
4.	Seedling height	60.322	10 48**	243	88.04
(C) Foliar characters (CTR&TI, Ranchi, Jharkhand 39 genotypes)					
1	Leaf area	58354.60	10.82***	16	2 16
2	Leaf length	126 1365	6 087***	1	0 13
3.	Leaf breadth	39.2317	13.796***	13	1 75
4.	L/B ratio	0.3805	4.376***	8	1 08
5.	L X B	92628 1331	10.0242***	4	0 54
6.	Single leaf weight	112.9532	102 0788***	448	60.46
7.	Frequency of stomata/unit area	222.1727	11.7568***	107	14.44
8	Length of stomata	0.0707	10.9113***	93	12 55
9.	Breadth of stomata	0 0283	7 5014***	51	6.88

*,**,*** : Significant at 5, 1 and 0.1% levels, L/B = Length · Breadth ratio

Table 10– Cluster means for fruit and seedling characters in 24 plus trees of *T arjuna* and *T tomentosa* complexes [43,44].

Cluster group	FRUIT CHARACTERS				SEEDLING CHARACTERS			
	Wt of fruit (gm)	Length of fruits (cm)	Width of wings of fruits(cm)	Germination %	Leaf length (cm)	Leaf width (cm)	L/B	Seedling height (cm)
I	4 71	5 61	0 96	78 50	8.440	2 939	3 032	16 396
II	1.63	3 36	0 86	69 33	8 941	2.181	3 932	22 260
III	3 45	4 99	1 32	72 44	8 421	2.920	2.960	14 730
IV	3.86	5 00	1.11	38 33	8 820	2 533	3 619	19 561
V	2.40	4 30	1.19	71.16	10 066	3.087	2 617	15 610
VI	4.45	4.76	1.30	66.83	6 610	2 257	2 927	10 070
VII	3 85	4 57	1 40	62.33	5.443	1 470	3 783	11.156
VIII	5.28	5.05	1 01	70 05	5.540	2 840	2 207	7 453
IX	2.87	3.74	0 60	72 00				
X	2.95	4 20	1 27	21.00				

(b) Diversity in half- sib seedlings

The half-sib seedlings of 22 naturally occurring hybrid trees and typical controls of *T. arjuna* and *T. tomentosa* were grouped into 8 clusters (Table 8).The seedling height, leaf length/leaf breadth ratio, leaf breadth and leaf length were found to contribute 99.04, 5.8, 3.62 and 2.544% respectively towards divergence (Table 9). The intercluster values of D^2 ranged from 2.638 to 18.148 suggesting wide diversity between the populations. Geographical diversity did not confirm the genetic diversity as the genotypes from the same region entered into different clusters. The cluster means (Table 10) further confirmed existence of very wide variation in seedling characters. Canonical analysis grouped the 24 genotypes into 10 clusters but confirmed the genetic diversity measured by D^2 statistics[44].

(c) Foliar diversity in 39 superior genotypes

The 39 genotypes of *T arjuna, T. tomentosa* and their hybrids fell into seven clusters (Table 11). The maximum contribution (60.46%) towards divergence was made by leaf weight followed by frequency (14.44%) and length (12.55%) of stomata (Table 9). The maximum (464.2) and minimum (107.667) cluster means for leaf area were exhibited by clusters VI and III while maximum (23.978) and minimum(2.49) cluster means for leaf weight, were exhibited by cluster VI and IV respectively (Table 12). Present studies support the view that separate taxonomic status is required for *T arjuna* var. *arjuna, T. arjuna* var. *augustifolia, T. crenulata, T. coriacea* and *T. alata* var. *alata.* Further , these studies also indicated that though *Terminalia* may have polytopic origin due to its three proposed centres of origin viz. Indo-Malaya, Africa and Australia, the centre of origin of section *Pentaptera*, which comprises of *T. arjuna* and *T. tomentosa* species complexes, lies in Central India due to occurrence of enormous genetic variability in this region [45].

Table 11–Clustering pattern of 39 genotypes of *Terminalia arjuna* and *T tomentosa* complexes [45]

Cluster (No. of plus trees)	Plus trees in the cluster	Species/Variety/Hybrid
I	14	*T arjuna* var *arjuna*
(24)	19	Hybrid
	18	*T arjuna* var. *arjuna*
	8	*T arjuna* var *angustifolia*
	29	Hybrid
	28	--do-
	13	--do-
	9	*T arjuna* var. *arjuna*
	12	Hybrid
	32	*T crenulata*
	31	Hybrid
	30	--do--
	16	*T arjuna* var. *arjuna*
	26	Hybrid
	17	*T arjuna* var. *arjuna*

Table 11 Contd .

Table 11 Contd .

	10	--do-
	15	*T. crenulata*
	35	*T coriacea*
	5	*T arjuna* var. *angustifolia*
	3	Hybrid
	11	*T arjuna* var. *arjuna*
	33	--do--
	6	--do--
	1	--do--
II	23	*T alata* var *alata*
(8)	38	-do-
	25	*T coriacea*
	34	-do-
	37	*T alata* var *alata*
	39	-do-
	24	-do-
	20	-do-
III	7	Hybrid
(1)		
IV	2	-do-
(1)		
V	27	-do-
(1)		
VI	21	*T alata* var. *alata*
(3)	36	*T coriacea*
	22	*T. alata* var. *alata*
VII	4	Hybrid
(1)		

Tocher cut off value 35.60

Table 12– Cluster means of foliar characters and their contribution towards divergence in 39 plus trees of *Terminalia arjuna* and *T. tomentosa* complexes [45].

Cluster	Leaf area $(cm)^2$	Leaf length (cm)	Leaf breadth (cm)	L/B	LxB	Single leaf wt (gm)	Stomatal frequency/ unit area	Stomatal length (μ)	Stomatal breadth (μ)
I	185.222	23.761	9.811	2.424	237 720	5.025	39.361	28 00	16 200
II	449.085	34 213	16 533	2.069	579.152	14 229	34 208	31.188	14.125
III	107.667	19.267	6.633	3.013	130.657	7.747	40.000	27.00	18.00
IV	142.667	18.400	8.567	2 263	166 343	2 490	56 667	26.00	22 00
V	176.667	22.467	10.00	2.237	226 300	10.443	25.333	28 50	16.50
VI	464.222	35.433	17.004	2.107	579 344	23 978	35.444	27.00	13.16
VII	73.333	24.300	8.933	2 700	219.647	6.220	67.333	25.00	18.00

(G) Cytology

The cytological studies conducted so far in *Terminalia* species[46-64] confirmed that x=12 is the basic number for the genus (Table 13). In *T. chebula* diploid, tetraploid and hexaploid cytotypes were recorded. The diploids are confined to Northern India, whereas all the three cytotypes are represented in the Central Indian forests. On the contrary, majority of the populations from north India are tetraploid and show abnormal meiosis in *T. bellerica* and the increase in size of various vegetative and floral parts is correlated with increase in ploidy level [65]. The polyploids appear to be of the allopolyploid type [50,64]. Gill *et al.* [67,68] suggested that allotetraploid and allohexaploid of *T. chebula are* segmental allotetraploid (AAA'A') and segmental allohexaploid (AAA'A'BB). According to them [68] the basic number of *Terminalia* is x=12, but *T. arjuna, T. bellerica* and *T. chebula* have also been found to show intra-specific aneuploidy with 2n=24 or 26. Sen [62] also reported 2n=26 for above three species. The 2n=14 as reported by Nanda [56] in *T. tomentosa* and *T. chebula* and other reports like x=7 and x=13 have been regarded as erroneous [69]. The tetraploid plants reported in *T. bellerica* and *T. chebula* exhibited a correlation between tannin content and polyploidy[51]. Recently, autotetraploidy, in addition to diploidy has been observed in *T. paniculata* also[70]. Chromosomes varied in length from 2.4-7.8μ in *T. arjuna*[69].

Karymorphological and meiotic details studied in six species revealed that the somatic chromosome complements consist mainly of long (more than 4μm) and medium (2-4μm) chromosomes. The genus possesses symmetrical karyotypes with either metacentric or submetacentric chromosomes. Each species seems to have its own karyotype regarding the position of centromere and arm ratio [71]. Meiosis was normal in all the six species. It appears that polyploidy and structural alterations in chromosomes have played their role in evolution of the genus *Terminalia* [72].

Table 13– Chromosome numbers in *Terminalia* species [47,48,53].

Species	Chromosome Number (2n)	References
arjuna W & A.	24	Janaki-Ammal & Sobti [51], Khosla & Kaur[69], Khosla & Sareen [52], Gill *et al* [49,67,68]
	26	Sen[62]
bellerica Roxb.	24	Mehra & Khosla[55], Mehra[65]
	26	Sen[62]
	48	Janaki-Ammal & Sobti[51], Munirajappa & Jayaramaiah[71, 72], Gill *et al* [67,68]
catappa Linn.	24	Simmonds [63], Sen [62], Riley [60], Chuang *et al.*[46], Munirajappa and Jayaramaiah [71, 72]
chebula Retz	14	Nanda [56]
	24	Janaki-Ammal & Sobti [51], Mehra & Khosla [55], Mehra [65], Gill *et al* [50,67, 68]
	26	Sen [62]
	48	Janaki-Ammal and Sobti [51], Gill *et al* [50], Munirajappa & Jayaramaiah [71,72]
	72	Gill *et al* [50, 67, 68].
crenulata Roth	24	Mehra [65], Mehra & Khosla [55]
ivorensis A. Cher.	24	Mangenot & Mangenot [54]

Table 13 Contd...

Table 13 Contd ..

muelleriana	24	Janaki-Ammal & Sobti [51]
myriocarpa Heurck & Muell	24	Mehra & Khosla [55]
oliveri Brandis	24	Singhal *et al* [64]
paniculata Roth	24	Munirajappa & Jayaramaiah [71, 72]
	48	Srivastav *et al.* [70]
phanerophlabia Engl et Diels	36	Riley [60]
tomentosa W. & A	14	Nanda [56]
	24	Janaki-Ammal & Sobti [51], Sanjappa & Rajagopalan [61]
	48	Mehra [65]

(H) Nuclear DNA contents

2C nuclear DNA amounts and fruits were studied in 37 samples collected from 27 mother trees of *T. arjuna* and *T. tomentosa.* Significant differences were observed between various taxa of *Terminalia* (Section:*Pentaptera*) both in 2C nuclear DNA amounts and fruits characters. The divergence and evolution in section *Pentaptera (T. arjuna* and *T. tomentosa* complexes) was accompanied by very high as well as highly variable nuclear DNA amounts ranging from 9.01 to 9.66 pg. The element of discontinuity in the distribution of DNA changes between complements was quite regular. The various taxa altogether fell into six cluster groups with an interval of 0.31 pg. between the two adjoining groups whereas various genotypes of *T. glabra* and spontaneous hybrids fell into five and four cluster groups with the interval of 0.3 and 0.51 pg., respectively (Figs. 1 and 2). This is probably the second case after *Cassia* where significant intraspecific nuclear DNA variation has been observed in trees [73]. In the light of hybridization [74] and nuclear DNA [73] studies besides D^2 analysis conducted for foliar [45] and fruit divergence [43], there is indeed a case for considering *T. glabra, T. berryi, T. coriacea, T. crenulata, T. alata* and spontaneous hybrids of section Pentaptera as separate species and not as the varieties of *T. arjuna* and *T. tomentosa.* The nuclear DNA amounts exhibited no correlation with fruit (seed) characters of various taxa studied [73].

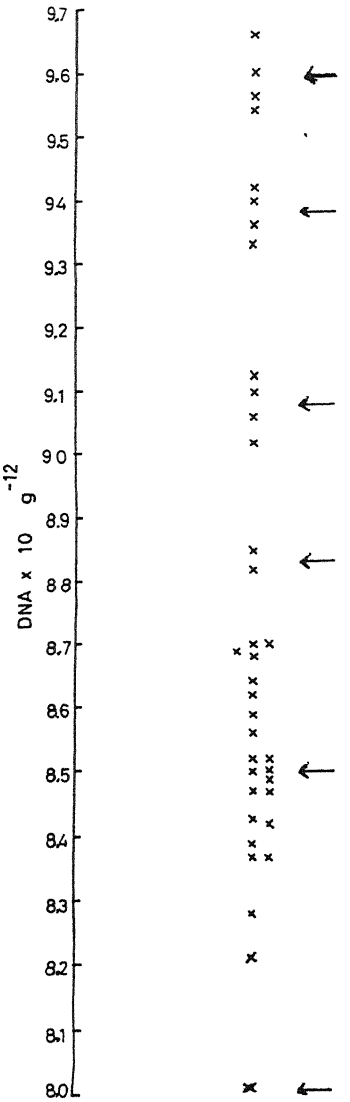

Fig 1–Mean DNA amounts in 37 Seed (fruit) types (27 mother trees) of *Pentaptera* Section. Note there is some indication of discontinuty in DNA values between groups of seed types.

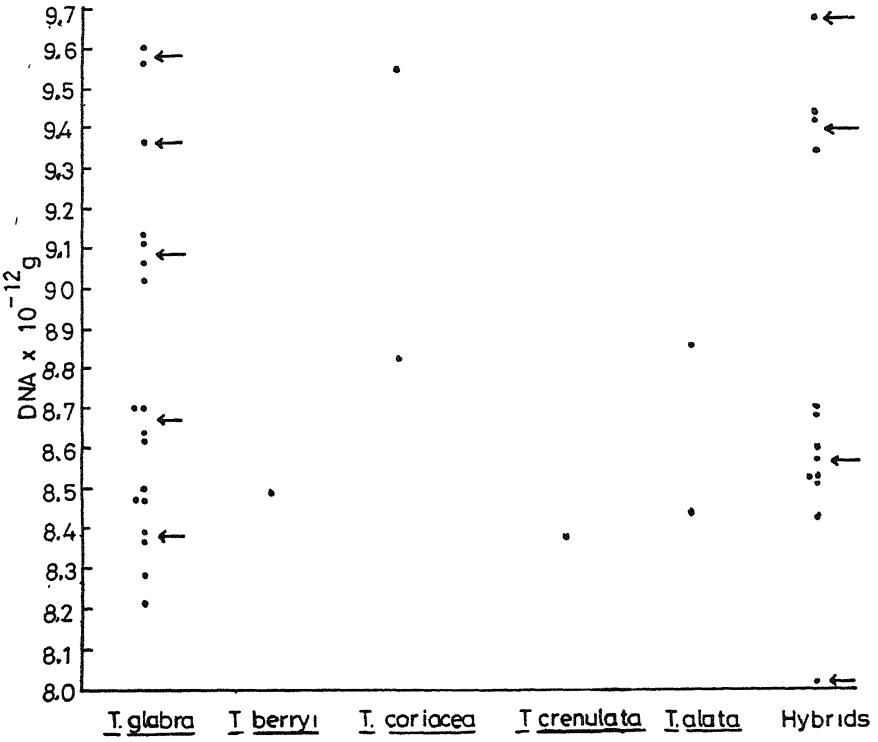

Fig 2–Mean DNA amounts in 37 seed types (27 mother trees) of *Pentaptera* section of genus *Terminalia* belonging to six taxa

(I) Vegetative propagation

(i) Macropropagation

For clonal propagation of desirable genotypes, airlayering was regarded as the most suitable method for *T. arjuna* [75]. Srivastava *et al.*[76] found that Indole Butyric acid at 200-300 ppm has given highest rooting percent (46-65%) and survival percentage (44-60%) in air layers of *T.arjuna* followed by IAA at 300 ppm which gave 61% rooting and 55% survival. Priya Ranjan *et. al.*[77] observed that genotypes belonging to *T. arjuna* var. *arjuna,* natural hybrids (between asan and arjun) and *T. crenulata* yielded best results with respect to airlayering in descending order. Mishra and Jaiswal[78] also found 60% rooting in *T. arjuna* airlayers raised with IBA at 20000 ppm.

Maximum rooting percentage (20%) in one year old stem cutting was recorded in IBA 1000 ppm followed by 12.4% rooting in IBA 500 ppm for 24 hrs treatment. The untreated cuttings did not respond to rooting[79]. During 1996-97, the leaf node cuttings yielded 31.63% rooting and 63.53% post transplantation survival while juvenile cuttings yielded 43.25% rooting and 97.38% survival in *T. arjuna* by keeping them in pit after planting in rooting media in polythene tubes [80].

During 1999-2000, the rooting time in *T. arjuna* was found to be minimum in juvenile cuttings (25.2 days) followed by leaf node cuttings (30.8 days), semi hard wood cuttings (33.6 days) and highest in hard wood cuttings (41.2 days) during favourable seasons of July-Sept. and April-June. Rooting response in juvenile cuttings taken from very young plants indicated 62.3% rooting with first node cutting and 50.8% rooting with second node cuttings in respect of *T. tomentosa* for the first time. During most favourable season of July-Sept., leaf node cuttings of *T. arjuna* required 25 ppm of IAA, IBA or NAA and yielded 86.6%, 76.6% and 66.6% rooting respectively as compared to 63.3% rooting in controls. On the contrary, semi hard and hard wood cuttings required higher concentrations of auxins (IAA 100 ppm, IBA 100 ppm or NAA 300 ppm) for induction of high rooting. The basal wounding induced 71.5% rooting in semi hard wood and 31.6% rooting in hard wood cuttings in *T. arjuna* against 8.33-16.5% rooting in control during July-Sept.. The rooting response further increased to 81.6% and 36.5% by application of IAA in semi hard and hard wood cuttings respectively. However, in *T. tomentosa* wounding could induce rooting only during Oct. - Dec. with 29.6% rooting in semi hard wood cuttings [81].

In *T. arjuna,* rooting in leaf node cuttings was further improved upto 97.5% against 61.6% and 19.2% rooting in Juvenile and semi hard wood cuttings respectively during April-June, 2000. Further improvements were achieved in *T. tomentosa* also where rooting enhanced upto 70% and rooting period reduced upto 25.4 days in leaf node cuttings and, juvenile cuttings exhibited as high as 65% rooting and rooting period as less as 21.4 days during April-Dec., 2000. Hence, leaf node cuttings are the most suitable material in *T. arjuna* as well as *T. tomentosa.* IAA, IBA and NAA improved rooting by 5-10% in leaf node cuttings at lower concentrations (25-75 ppm) in *T tomentosa.* Plants raised through saplings exhibited 52.7% higher leaf yield than those raised from seeds. The rootability of improved genotypes was found to be in the order of B_2 (43.06%) > D (16.6%) >N_5 (10%) > DS_2 (6.6%) which shows that differential response to rooting exists in *T. arjuna* [82]. Sinha *et al.* [83] identified machanical barrier in the form of sclerenchymatous and collenchymatous tissues as a factor hindering root emergence and achieved more than 86.6% root

emergence in *T.arjuna* by countering this hinderance either by using softer cuttings from young bushes or by partially removing hard tissues from cuttings.

In *T. arjuna* and *T. tomentosa* approach grafting has also been found useful for establishment of clonal orchards of superior trees at CTR&TI, Ranchi as mentioned under conservation.

The cuttings of *T. bellerica* planted during July, Oct. and March failed irrespective of the month, hormone treatment and portion of the cuttings. Hence, *T. bellerica* may be categorized as under obstinate to root under field conditions[84]. Mishra and Jaiswal[78] studied the effect of IBA at 2500-20000 ppm on the rooting of air layers of *T. chebula* and found that IBA 10000 ppm induced highest rootings (73%). The cutting of *T. chebula* planted in July and Oct. failed completely while in March the middle portion of the cuttings with combination of 4000 ppm of IBA produced better results[84]. Subsequently, Jose and Thomas[85] found that 100% of the juvenile cuttings placed in pots containing river sand in mist chamber exhibited rooting without any special treatments within 30 days of planting but air layers failed to root like stem and root cuttings from old trees. Ramesh Singh *et al.*[86] also achieved 67-78% root induction from stem cuttings of one and two year old juvenile seedlings of *T.chebula*. They also achieved success with peg grafting of presprouted stem cuttings and grafting of entire sprouts in *T.chebula* .

Cleft grafting has got success of 70% undermist chamber as compared to 100% success in approach grafting in *T. chebula*[87]. Besides patch budding has also been found successful in *T. chebula* [88]

(ii) Micropropagation

Studies on micropropagation of *T.arjuna* at CTR&TI, Ranchi revealed that maximum shoot development (50-60%) was observed in nodal explants in MS-media during 10-30 days in combination with NAA+IBA+BAP+KIN (0.01-2.0 mg./l.) supplemented with antioxidants and absorbents [89] whereas maximum root proliferation was observed in MS-media supplemented with IBA+BAP+KIN (0.50-2.5 mg./l.) + antioxidants + absorbents [90]. For shoot initiation and proliferation MS-media supplemented with BAP+KIN+CW+CH+AC (2%) and for root initiation MS-media supplemented with BAP+KIN+IBA (1.0-2.0 mg./l.) + Bio (0.1-1.0 mg./l.) were also found best. The four plantlets developed *in vitro* died during hardening in soil + sand + FYM and sand media after one month. The leaf explants were found to be most suitable for callus culture in *T.arjuna* and *T.tomentosa* [91]. Ramesh *et al.* [92,93] subsequently developed protocol for micropropagation of *T.arjuna* Bedd. through

axillary bud culture and reported that MS-media containing TDZ (0.05-1.0 mg./l.) showed early bud breakage (5-7days) with maximum shoot proliferation (1.89 ± 0.21) at 0.05 mg./l. while modified B_5 medium supplemented with IBA showed high quantity adventitious roots with laterals. The plantlets were transferred successfully to pots containing vermiculite mixture in green house for hardening for 3-4 months. Microprpopagation of *T. arjuna* has also been achieved from cotyledonary nodes by Pandey and Jaiswal [94].

(J) conservation

Pressure associated with rearing of tasar silkworms, over exploitation by leather, timber, and pharmaceutical industries, over grazing, indiscriminate felling and conversion of forest land into agricultural land and human settlements have led to fast erosion of genetic resources of *Terminalia.* Their fast depletion is also likely to disturb the ecological balance of the tropical forests. Hence, a concerted effort to conserve existing genetic diversity of the genus *Terminalia* are urgently required. Conservation of genetic resources of *Terminalia* species have been adopted through *in situ* as well as *ex situ* approaches:

(i) *In situ* conservation

The *in situ* conservation is being practised through national parks, scientific reservations, natural areas like Kanha National Park, establishment of seed production areas and plus/elite tree selection as detailed elsewhere [19,31,82,86,95-98].

(ii) *Ex situ* conservation

The *ex situ* conservation in *Terminalia* is being resorted through seed orchards, seedling banks and experimental plantings. In Arunachal Pradesh 3.0 ha. seed orchard of *T. myriocarpa* has been established by Forest Deptt. [95,96]. Seedling Banks of *T. arjuna* and *T. tomentosa* have been established in Central Tasar Research and Training Institute, Ranchi and its 5 Research Extension Centres, 4 Regional Tasar Research Stations and 19 Basic Seed Multiplication & Training Centres for tasar culture in Jharkhand, Chattisgarh, Orissa, West Bengal, Andhra Pradesh and Uttar Pradesh. The total area under natural *T.tomentosa* and raised *T.tomentosa* and *T.arjuna* through seedlings accounts to approximately 10.7 ha, 936.0 ha. and 631.8 ha. area respectively. Besides, Kosa Beej Kendra and Pilot Projects Centres under Sericulture Deptt. of various State Govt. have also raised seedling banks for tasar rearing [97,98]. 33 seedling and 14 clonal orchards of both the species are also being maintained at CTR&TI, Ranchi as experimental plantings[81]. Besides, a field gene

bank of *Terminalia* species has also been established at CTR&TI, Ranchi by using inter-specific approach/wedge grafting tachniques. At present, 129 accessions (*T arjuna* 80, *T.tomentosa* 34, *T.bellerica* 06, *T chebula* 07 and *T catappa* 02) have been planted under 150 x 240 cm spacing with 4 plants in each accession [99].

(iii) Strategies

Following strategies have been suggested by the author for conservation of *Terminalia* species [19,20,97,98].

1. A Non-mulberry Silkworm and Plant Germplasm Station should be established which should have different units at suitable places.
2. Greater emphasis should be laid for conservation of Kahvi from Bilaspur, Ulta Saja from Amarkantak and spontaneous hybrids from Central India since they are rare and endangered.
3. Small and big leaf forms of *T. crenulata* and *T. coriacea* should be introduced to other regions from Western and Eastern Ghats
4. Collaboration between I.C.F.R.E., B.S.I. and C S.B. Institutes should increase. Besides, different RTRSs should exhange their germplasms
5. *Terminalias* should be declared as national Trees.
6. Potentialities of *T. arjuna* for reclammation of alkaline/saline soil, water logged/peaty soils and ash dykes from thermal plants and *T. tomentosa* for reclammation of barren rocky lands should be publicised for plantation by Railways, Coal India Limited, National Thermal Power Corporation, Private industries and NGOs.
7. Ecological awareness should be generated among tribal populations.
8. Apart from institutes of Central Silk Board, TFRI (Jabalpur), KFRI (Peechi), FRI (Dehradun), B.S.I. (Shillong) and/or R&MDFRI (Jorhat) etc should also establish germplasm bank of *Terminalia* species.
9. Religious sanctity of Arjuna trees about birth of Arjuna,the legendary hero of Mahabharat in Arjuna forests, may be publicised among rural people to check their felling.
10. *In situ* conservation of *Terminalia* species should involve continued sustainable utilization and entail comprimises between their conservation and utilization.
11. Research on *in vitro* propagtion and conservation should be intensified as no remarkable success has been achieved in this direction.

The biopiracy of *T. arjuna, T. chebula* and *T. bellerica* is being attempted in some countries which should be taken care of to avoid national loss (PTI, Feb. 15, 98).

Tree Improvement

(A) Natural variations

Enormous variations encountered in species of *Terminalia* with respect to shape and size of leaves and fruits are of continuous nature rather than discontinuous nature. A strong inter-relationship between the number of sepals and stamens in flowers and number of wings in fruits have been observed in *T. arjuna* and *T. tomentosa*. While a flower with four sepals and eight stamens gave rise to a fruit bearing four wings, flower bearing ten sepals and twenty stamens gave rise to a fruit bearing ten wings. This close association between the number of sepals, stamens and wings may be either due to pleiotropism or close linkage of genes governing these characters. While sepals ranged from 4 - 12, stamens ranged from 8 - 24 in flowers and wings in fruits ranged from 4 - 12 in number. The number of stamens was found to be double to that of number of sepals in the flowers and the number of wings in fruits was found to be equal to that of number of sepals in the flowers from which they arose. The occurrence of 5 sepals, 10 stamens and 5 wings has been recorded in 95.277% cases followed by 4 sepals, 8 stamens and 4 wings in mere 2.944% and 6 sepals, 12 stamens and 6 wings in only 1.222% cases. While the cases of 7 - 12 sepals and wings with corresponding 14 - 24 stamens were very rare (0.055 - 0.166%), the cases of 3, 9 and 12 sepals and wings with corresponding 6, 18 and 24 stamens exhibited minimum (0.055%) frequency. The progenies from the fruits bearing 4-10 wings have been raised [4].

In *T. chebula* various grades of fruits have been earmarked on the basis of locality/type viz. Bhimlies-Madras (T.N.), Jubbulpores-Jabalpur (M.P.), Rajpores-Kolhapur (MS), Vingorlas-Bombay (MS), Madras Coast, Salem-I (T.N.), Salem-II (T.N.), Survari, Bala and Java harde etc. Furthermore, six kinds of fruits of *T.chebula* viz., Halileh-i-zira (Coumin seed size), Halileh-i-javi (Barley corn size), Halileh-i-zangi (Raisen size), Halileh-i-chini (greenish yellow and hard), Halileh-i-asfar (nearly matured) and Halileh-i-Kabul (fully matured) have also been recognized for medicinal purposes [1]

(B) Aims and objectives

The following aims and objectives for breeding of *T. arjuna* and *T. tomentosa* species are taken into consideration from tasar culture point of view:

(1) Isolation/evolution of protein rich superior genotypes with quality foliage.

(2) Isolation/ evolution of fast growing and high leaf yielding genotypes.

(3) Isolation/evolution of dwarf and bushy genotypes.

(4) Isolation of stem borer and gall resistant genotypes.

(5) Isolation of drought resistant genoytpes.

(6) Isolation/evolution of heterotic genotypes.

(7) Isolation / evolution of polyploid genotypes.

These objectives will lead to an increase in the production of tasar / unit area, improvement of tasar quality, reduction in the expenditures incurred on insecticides / pesticides and fertilizers, minimization of pollution due to these chemicals and extension of tasar culture in arid /semi-arid regions of the country.

On the contrary, breeding of *T.chebula*, *T.bellerica* and *T.catappa* is oriented towards improvement of size and quality of fruits either due to their importance in pharmaceutical industries or due to their edible nature.

(C) Candidate/plus tree selection

The objectives of such ventures have been identification, conservation and utilization of genetic resources of *T. arjuna* and *T. tomentosa* for tasar culture. Their genetic resources were surveyed at various places of Jharkhand, Orissa and Chattisgarh during 1987, 1989, 1990 and 1993 in four explorations and 131 plus trees were identified. Out of these, 50 plus trees were selected from the artificially cultivated populations of *T. arjuna* and *T. tomentosa* at Central Tasar Research and Training Institute, Piska Nagri, Ranchi (Jharkhand) whereas 66 and 15 plus trees were selected from the forests of Chattisgarh and Orissa respectively (Table 14). Fifth exploration was conducted in Maharashtra during 2000 and sixth exploration was conducted during 2001 in Uttaranchal as a result of which 17 plus trees of *T. arjuna,* 16 plus trees of *T. tomentosa,* 5 plus trees of *T. bellerica* and 4 plus trees of *T. chebula* were also selected by C.T.R.&T.I.[82]. Besides, 10 phenotypically superior trees of *T.tomentosa* were also selected by Karoshi and Patil in 2000 [31].

41 plus trees of *T.myriocarpa* were selected in Arunachal Pradesh for forestry purposes by forest department[96] and 13 plus trees of *T. chebula* were selected in Haryana for exceptionally large fruits in greater quantity for medicinal purposes by forest depatment in collaboration with Punjab Universitty, Chandigarh [86] .

(D) Progeny testing and elite trees

Progeny testing and field trial of 22 plus trees of *T. arjuna, T. tomentosa* and their spontaneous hybrids revealed that progeny testing during nursery provides preliminary informations as ranking of progenies may change in the field. The correlation between seedling characters during nursery and yield and yield contributing characters during field trial indicated that seedlings exhibiting higher leaf length, lesser leaf breadth and greater height should be selected for plantation so as to increase leaf productivity per unit area which will inturn increase rearing capacity and hence cocoon production per unit area [100]. Further correlation studies between seed characters and plant characters under field trial revealed that seeds of only those candidate trees should be selected which exhibit less breadth of wings and high germination percentage as plantations raised from them ensures high productivity of leaves per unit area for tasar silkworm rearing [101]. From leaf yield point of view, progenies of Ds_3, N_6, Ds_4, S_2, S_3, S_1, O2 and O1 plus trees exhibited superiority in descending order. Hence, they may be considered as elite trees.

(E) Provenances and their trial

Informations on provenances and their trial are lacking in *T.arjuna* and *T tomentosa* due to indescriminate collection of seeds from adjacent forests and raising of seedling orchards by Central Silk Board and state government units for tasar culture in various states. However, three provenances of *T. chebula* viz. Brahmpura, Sahalyo and Mandhana were recently selected in Haryana state in Yamuna Nagar and Panchkula districts. Base population of 16, 2350 and 180 trees are existing in these provenances respectively. Pollen transfer barrier exists among these three discrete populations due to separations caused by successive hill ranges and physical distance. Evaluation of yield of Harad fruits during 1999-2002 revealed that highest yield of 16.87 qtls./plant/year was exhibited by Brahmpura provenance while lowest yield of 0.62 qtls./plant/year was exhibited by Sahalyo provenance[86].

(F) Seed stand/seed orchard

The seed stand/seed production areas of *T. myriocarpa* and *T. arjuna* species were established in 1.0 ha and 3.0 ha. in Arunachal Pradesh and Uttar Pradesh respectively. The seed orchard of *T. myriocarpa* has been developed in 2 ha. in Arunachal Pradesh[96]. Central Tasar Research & Training Institute, Ranchi has established 14 clonal orchards of screened varieties of *T. arjuna* and hybrids of *T. arjuna* and *T. tomentosa*. Besides, 33 seedling orchards of these taxa are also being maintained there for evaluation and isolation of superior varieties for tasar culture[81].

(G) Hybridization

During 1989-90, interspecific crosses between *T. arjuna*, *T. chebula* and *T. tomentosa* failed barring *T. tomentosa* x *T. arjuna* cross which succeeded partially since only 10 indehiscent fruits developed but they dried after two months probably due to endosperm incompatibility. During 1991-92, six crosses involving six superior genotypes of *T. arjuna* and *T. tomentosa* complexes were made which resulted into setting of 250 indeshiscent fruits out of which only 10 interspecific and 10 intraspecific fruits finally matured but they failed to germinate. During 1992-93, only 12 fruits of hybrid nature finally matured but they also failed to germinate while no fruits from various crossings matured during 1993-94. These studies suggest that in order to produce hybrids, *T. tomentosa* genotypes should always be used as female parents[74].

(H) Polyploidy and mutation breeding

Out of 450 seedlings of *T. arjuna* and *T. tomentosa* subjected for colchicine (0.15%) treatment during 1990-92 only 10 plants behaved like polyploids. Cytological investigations are required to confirm their ploidy level and chromosome behaviour during meiosis[102,103].

6000 seedlings of both above species were subjected for EMS and MMS (0.1 & 0.15%) treatment during 1991-92 to evolve fast growing, high yielding, protein rich, bushy genotypes. 0.12% EMS was determined as LD-50 for *T. arjuna* seedlings. Some of the treated seedlings have shown bushy nature from the beginning. Further, studies are under progress at CTR&TI, Ranchi[104,105].

The rediosensitivity was also studied by irradiating *T. arjuna* seedlings with gamma rays . The percentage germination and early survival were drastically reduced at 5-60 K rad and various types of abnormalities in leaf and growth characters were observed in treated plants at 40 K rad which was determined as LD 50 for *T. arjuna*. It was suggested that improved survival and growth found at some doses of irradiation could be used to improve fuel wood crops like *T. arjuna*[106].

(I) Breeding for drought resistance

Initial screening of 39 genotypes of *T. arjuna* and *T. tomentosa* from Ranchi for stomatal frequency and length and breadth of stomata have revealed that there is nearly three fold variation in stomatal frequency and two and half fold variation in size (LxB) of the stomata[45]. Such studies may be carried further to evolve drought

resistant genotypes in conjunction with proline status of various genotypes at CTR&TI, Ranchi.

(J) Breeding for fast growth and early sprouting:

Early sprouting in many pruned plants of equal age in *T. arjuna* and *T. tomentosa* has been noticed at CTR&TI, Ranchi. Accordingly, screening of early sprouting plants has been made continuously for two years in the same plantation. For few more year, such screening will be required for confirming their faster growth rate. Seedling growth in progenies of various plus trees followed by estimation of overall dry mass may also help us in identification of the fast growing genotypes. Investigations along these lines in conjunction with induction of polyploidy, mutagenesis and hybridization programmes are likely to yield desirable results. Fast growing genotypes of *Terminalia* species will not only reduce the gestation period but they will also enable sericulturists to take three crops annually from the same plantation.

Table 14– Plus trees of *T arjuna* and *T tomentosa* selected by CTR&TI, Ranchi for tasar culture [94]

Sl No	Year of selection	No. of plus trees	Designation of plus trees	State in which located	Detailed location
1.	1987	6	B1-B6	Orissa	Bijlikhaman, Sundargarh
2	1987	2	O1-O2	Orissa	Opposite BSM&TC Office, Sundargarh
3.	1987	3	S1-S3	Orissa	Samradihi, Sundargarh
4	1987	4	N1-N4	Chattisgarh	Along Raipur-Jagdalpur Road, Bastar.
5	1987	2	N5-N6	Orissa	Umerkot.
	1989	2	N7-N8		Nowrangpur.
6.	1987	1	D	Chattisgarh	Dhamtari
7.	1987	4	DS1-DS4	Chattisgarh	Sorgaon, Dhamtari
8	1993	4	PB1-PB4	Chattisgarh	Parastola, Balaghat
9.	1993	4	VB1-VB4	Chattisgarh	Near Balaghat River, Balaghat.

Table 14 Contd...

Table 14 Contd

10	1993	4	JB1-JB4	Chattisgarh	BSM&TC Farm, Bastar
11	1993	1	JR1	Chattisgarh	Kosa Beej Kendra, Chaparbhanpur, Bastar
12.	1993	4	JC2-JC5	Chattisgarh	Near Chitrakot Water Fall, Bastar
13	1993	1	JR1	Chattisgarh	Opposite Silk Farm, Nawagaon, Bastar
14	1993	5	JR2-JR6	Chattisgarh	Along Jagdalpur- Raipur Road, Bastar
15	1993	2	BP1-BP2	Chattisgarh	Along Bilaspur-Pendra Road (Amarkantak)
16	1993	3	BPI-BPIII	Chattisgarh	BSM&TC Farm, Pali, Korba.
17	1993	1	BPIV	Chattisgarh	Kosa Beej Kendra, Pali, Korba
18	1993	1	BK7	Chattisgarh	ISTP Plantation, Dhelwadih, Korba.
19	1993	6	BK1-BK6	Chattisgarh	REC, Rampur Farm, Katghora, Korba
20.	1993	5	RB1-RB5	Chattisgarh	BSM&TC, Boirdadar Farm, Raigarh.
21	1993	10	RL1-RL10	Chattisgarh	CTSSS, Lakha Farm, Raigarh.
22.	1993	6	RLL1-RLL6	Chattisgarh	Kosa Beej Kendra/PPC Lailunga, Raigarh
23	1990	39	PBG1-PBG39	Jharkhand	CTR&TI Farm, Ranchi
24	1993	11	PBG40-PBG50	Jharkhand	CTR&TI Farm, Ranchi

Effect of tree improvement on

(a) Tasar quality

Foliar nutrients influence the health and growth of larvae, quality of silk and overall silk production since most of the amino acids and minerals are directly derived by the silkworms from their food plants. Hence, nutritionally rich genotypes were searched for various foliar nutrients viz. aminoacids, minerals, crude fibre, total nitrogen (protein) and carbohydrates (glucose/fructose) through A.O.A.C. and Kjeldahl methods at CTR&TI, Ranchi which revealed following facts:

(i) Nonhairy *T. tomentosa* is nutritionally more superior than hairy *T. tomentosa*[107].

(ii) Out of eleven spontaneous hybrid plus trees from Sundargarh and two typical controls of *T. arjuna* and *T. tomentosa*, the overall performance of S_1 and O_1 trees is superior in respect of various foliar nutrients and quantitative foliar characters [108] (Tables 15,16).

Table 15– Mean values of leaf characters in various plus trees of spontaneous hybrids in *Terminalia* (Section . *Pentaptera*) [108].

Place/plus trees	Leaf characters				Scoring for LxB
	Length (cm)	Breadth (cm)	L/B	L x B (cm^2)	
CTR&TI,Ranchi					
T arjuna	12.80	4 63	2.74	59.89	(12)
T tomentosa	24.90	9.80	2.67	412.60	(1)
Sundargarh(Orissa)					
B1	14 03	7.57	1 82	112.25	(6)
B2	11 30	5.50	2 07	63.83	(11)
B3	13 60	6.80	1.99	92.85	(8)
B4	12 57	4 47	2 79	56 99	(13)
B5	13.87	5.83	2 39	80.63	(10)
B6	17.93	10.17	1.77	185.61	(2)
S1	19.15	6.30	3.05	122 74	(4)

Table 15 Contd

Table 15 Contd .

S2	14 13	6 37	2 20	90 90	(9)
S3	17 10	6 53	2 60	121 21	(5)
O1	20 53	6 90	2 92	146 25	(3)
O2	14 23	6 53	2 15	94 61	(7)
General mean	15 858	6 723	2 396	126.182	
C D. at 5 %	6 97	2.48	0 67	181 65	
Significance (F)	2.62*	3.95**	3 44**	2 25*	

*- Significanct at 5 % level; ** - Significant at 1 % level

Table 16– Mean values of foliar constituents and ranking in various plus trees of spontaneous hybrids of *T arjuna* and *T tomentosa* [108]

Place/plus trees	Total foliar constituents (%)					Pooled total scoring	Ranking
	Minerals	Crude fibre	Moisture	Carbohy-drates	Nitrogen		
CTR&TI Ranchi (Bihar)							
T arjuna	12.20(6)	17 50(7)	70 50(2)	6 50(9)	2.59(3)	39	VII
T tomentosa	9.80(10)	17.70(8)	69 33(6)	5 04(11)	1 96(7)	43	X
Sundargar (Orissa)							
B1	10.20(9)	17.10(6)	68.33(8)	6 64(8)	2.31(5)	42	IX
B2	6.20(11)	15.60(1)	70.33(3)	6.97(5)	3 50(1)	32	V
B3	8.00(12)	16 73(4)	68 67(7)	6 80(7)	2.38(4)	42	IX
B4	11.60(7)	17.70(8)	68.67(7)	8 06(2)	2.59(3)	40	VIII
B5	10 20(9)	16.20(2)	70.07(4)	7.08(4)	2.87(2)	31	IV
B6	12.40(4)	16.80(5)	69.33(6)	6.30(10)	1.55(10)	37	VI
S1	13.70(3)	16.80(5)	70.33(3)	4.45(13)	3.50(1)	29	II
S2	12.30(5)	16.20(2)	69.33(6)	4.87(12)	2.10(6)	40	VIII
S3	11.00(8)	15.60(1)	71.67(1)	8.32(1)	1.68(9)	25	I

Table 16 Contd.

Table 16 Contd

O1	15 30(2)	16 20(2)	67 67(9)	7 98(3)	1 54(11)	30	III
O2	17 3(1)	16 30(3)	70 00(5)	6 89(6)	1 80(8)	30	III
General mean	11 785	16 649	69 556	6 678	2 337		
C D at 5%	1 05	1 16	2 67	1 02	0 08		
Significance(F)	50 48***	3 22**	1 38^{NS}	13 24***	623.13***		

NS - Non significant, **,*** - Significant at 1 0 and 0 1 % respectively, Values in parentheses are ranking with respect to the particular foliar constituent

(iii) Plus trees of putative hybrids, *T glabra* and *T. crenulata* are more superior nutritionally, yet, genotypes of *T. alata* var. *alata* and *T. coriacea* attain higher ranking and overall superiority due to their higher leaf size/area and drought resistance chracters [109].

Evaluation for rearing performance of nutritionally superior genotypes (plus trees) and their effect on improvement of quality of tasar silk with respect to silk filament, reelability, denier, neatness and elasticity etc. needs to be taken up in near future.

(b) Tasar quantity:

Evaluation of 22 progenies raised from seedlings of plus trees from Chattisgarh, and Orissa and 50 plus trees at CTR&TI, Ranchi is being carried out. The rearing performance of 22 progenies studied during 1991-92 to 1996-97 revealed that mean of ERR and cocoon weight ranged from 67.25-79.44 and 13.95-14.46g respectively. The mean of larval and shell weight, SR% and absolute silk yield ranged from 34.37-37.979g, 1.68-1.97g, 12.37-13.66% and 99.03-124.32g respectively. On the basis of overall rearing performance (absolute silk yield), progenies of N_6, D_{S2}, D, N_5, S_1 , B_3, D_{S4}, B_2, B_5 and D_{S3} plus trees have been found superior in descending order. However, from leaf yield point of view half-sib progenies of D_{S3}, N_6, D_{S4}, S_2, S_3, S_1, O_2 and O_1 plus trees exhibited superiority in descending order. When leaf yield as well as silk productivity is considered jointly, half-sib progenies of N_6, D_{S3}, S_1, D_{S4}, N_1, B_6, N_5, D, D_{S2} and D_{S1} plus trees exhibited superiority in descending order for rearing [110]. Therefore, these plus trees may be considered as elite trees. The overall superiority percentage of these elite trees was found to be 78.54, 71.97, 67.77, 67.4, 55.75, 54.6, 42.26, 39.78 and 32.18 respectively with respect to lowest yielder.

(C) Other aspects

Studies on provenance trial and candidate plus trees of *T.chebula* indicated that production of Harad fruit may be increased by 2621% from 0.62 qtls./plant/year to 16.87 qtls./plant/year [86]. Such economic beneficial genetic gains may be transferred to prospective growers in case further studies are carried out in other medicinal species like *T.bellerica* and fruit species like *T.catappa* (Jungali Badam) also.

Conclusion

Genus Terminalia should be divided into four sections viz., *Catappa*, *Myrobalanus*, *chuncoa* and *Penatptera* and separate taxonomic status may be assigned to *T. glabra* (*T. arjuna* var. *arjuna*), *T. berryi* (*T. arjuna* var. *angustifolia*), *T. crenulata*, *T. coreacea* and *T. alata*. Centre of origin of these species (section *Pentaptera*) lies in Central India. Geographical diversity does not correspond to genetic diversity and fruit weight, seedling height and leaf weight contributed maximum towards genetic divergence in *Terminalia*. Pollination is entomophillous and floral biology of *T. arjuna* is much closer to *T. tomentosa* than to *T. chebula* and *T. paniculata*. For hybridization, genotypes of *T. tomentosa* should be used as female parents. Basic number for genus *Terminalia* is x = 12. In *T. bellerica* and *T. paniculata* diploid and tetraploid and in *T. chebula* diploid, tetraploid and hexaploid cytotypes were recorded. Polyploidy, structural alterations and nuclear DNA amounts in chromosomes and hybridization together led evolution in genus *Terminalia*.

Leaf node cutting are most suitable material for clonal propagation in *T. arjuna* (97.5% rooting) and *T. tomentosa* (70% rooting). Approach grafting is also highly suceesful for establishment of clonal orchards in these species. While *T. bellerica* is obstinate to root, juvenile cuttings of *T. chebula* exhibit rooting (100%) without any treatment. Micropropagation of *T. arjuna* has been found successful through axillary bud culture.

Conservation of genetic resources of *Terminalia* have been started through *ex situ* as well as *in situ* approaches which may be fastened through establishment of a nodal germplasm station and nationalisation of genus *Terminalia*.

In order to avoid unwanted duplications in germplasm of *Terminalia* species, biomolecular characterization of genotypes should be carried out through RAPD, RFLP, AFLP, SSR, ISSR and EST since they are more reliable.

Intra-specific variations in fruit characters of medicinal species and foliar characters in host species of tasar silkworms in genus *Terminalia* may be utilized to

enhance production due to their heritable nature. Leaf yield / plant, No. of branches and No. of leaves / branch, height of the plants , net photosynthetic rate, chlorophyll, protein and starch content of leaves should be considered as effective parameters for selection in *T arjuna* to increase tasar silk production / unit area. For increasing productivity of tasar silk, plantations should be raised only from seeds of those candidate trees which exhibit less breadth of wings and high germination %.

Stomatal studies may be carried further alongwith proline status to evolve drought resistant genotypes for desert / semi-arid regions while breeding for fast growth shall not only reduce gestation period but may also enable tribals to take up three crops annually from the same plantations of *T. arjuna* and *T. tomentosa.* Genotypes of putative hybrids, *T. glabra* and *T. crenulata* are found nutritionally superior for tasar silkworms yet genotypes of *T alata* and *T coreacea* are found better for drought / rocky areas.

While effect of plus/elite trees on quality of tasar silk needs to be ascertained, half-sib progenies of N_6, D_{S3}, S_1, D_{S4}, N_1, B_6, N_5, D, D_{S2} and D_{s1} elite trees exhibited overall superiority of 78.54, 71.97, 67.77, 67.4, 55.75, 54.6, 42.26, 39.78 and 32.18 percent respectively when leaf yield as well as absolute silk yield are considered jointly. Improved survival and growth rate found at certain doses of irradiation could be used to improve fuelwood of *T arjuna.* The production of Harad fruits may be increased by 2621% through elite trees in *T. chebula.* The clonal orchards of elite trees should be evaluated and plantations be raised only from their clones in future to transfer maximum economic benefits to prospective cultivators.

Molecular breeding for introgression of genetic markers associated with disease/pest resistance or tolerance may be attempted through Marker Assisted Selection. Map based cloning and transposon tagging methods may also be employed to isolate genes corresponding to desirable traits and genetic engineering tools may be employed for incorporation of Nif genes in *Terminalia* species in future

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Structural time-series modelling for describing India's milk production data

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Abstract

During the last two decades or so, all – India milk production has witnessed a rapid growth We are now in a position to export milk products to other countries In this article, a new promising approach of "Structural time-series modelling" is discussed and applied to describe the path of our country's milk production based on 1981 - '98 data. The identified model is then used to make forecasts for the next five years

(Keywords: logistic growth model/milk production data/STAMP software package/structural time-series modelling)

Introduction

It is well recognized that Indian dairy industry makes a substantial contribution to the Gross National Product (GNP) from agriculture. In terms of milk production, it is the third largest industry in the world. Several studies, dealing with various aspects of "Dairy industry" have been carried out in the last decade (Chand *et al*[1]., Gandhi and Mani[2], Patel[3]). The recent WTO's regulations have clamped down heavily on subsidies, and this has resulted in a boon for exporting Indian dairy products. A substantial demand abroad for Indian milk products is recently emerging in countries, like Holland, Russia, and Kuwait. Thus it is of great relevance to develop an appropriate statistical model for forecasting all - India milk production, which would help in formulating efficient policies.

Balain *et al.*[4], among other things, projected the all - India milk production for 2000 A.D. on the basis of simple growth rate pertaining to the period 1981 to 1990. The methodology adopted does provide useful information but its limitation is that it assumes a "linear" rate of growth that can evidently be sustained only for a short

period of time. Accordingly, purpose of the present paper is to discuss "Structural time-series models" for modelling and forecasting all - India milk production data.

Method

Various Statistical Models

With a view to describing all - India milk production data, the following statistical models are discussed:

1. *Logistic growth model (LM).* This model is widely employed to describe the dynamics of a growth process and is given by the equation

$$Y_t = C / (1 + be^{-at}) + \varepsilon_t , \qquad t = 1, 2, ..., T \tag{1}$$

where Y_t is the all - India milk production at time at t; 'a' is the intrinsic growth rate, 'C' is the carrying capacity, 'b' is $C/Y_1 - 1$, and ε_t is a normally distributed, independent random disturbance term with mean zero and constant variance σ^2, i.e. $\varepsilon_t \sim N (0 , \sigma^2)$. As the parameters in eq. (1) are appearing in a nonlinear manner, these can be estimated by using "Nonlinear estimation procedures" available in Statistical Analysis System (SAS[5]) software package. One of the main assumptions about error term is that these are independent and this can be examined by using the well-known "Run test". If this assumption is violated, the model with AR (1) errors given by

$$\varepsilon_t = \varphi \varepsilon_{t-1} + \eta_t, \quad |\varphi| < 1, \tag{2}$$

can be fitted using PROC ARIMA module available in SAS package. It may be mentioned that similar approach was adopted by Man – Molinero[6] for forecasting number of tractors in Spain.

2. *Structural time-series model for flow (STMF) :* Recently, a very promising approach of "Structural time-series modelling" (Harvey[7]) has been developed to analyse time-series data. Here the model is set up in terms of components of interest, such as trend (μ_t), seasonal variation (γ_t), cyclical fluctuation (Ψ_t), and an error term (ε_t). In the presence of trend and error term, the model reduces to

$$y_t = \mu_t + \varepsilon_t, \qquad t = 1, 2, ...,T \tag{3}$$

where y_t, the net increase, is $y_t = \Delta Y_t = Y_t - Y_{t-1}$, $t = 2, ..., T$ and ε_t follows N (0 , σ_ε^2). In the subsequent discussion, following Harvey[8], it is assumed that the net increase is always positive, i.e. $y_t > 0$. This is a reasonable assumption although it would obviously not be appropriate in all cases where logistic growth model has been used. As the underlying time-series is assumed to be increasing as time progresses, it cannot have a 'Cyclical component'. Let the trend in eq. (1) be denoted by the continuous function (μ_t), i.e.

$$\mu(t) = C/(1+b\, e^{-at}),\ 1 \le t \le T \tag{4}$$

Differentiating, re-arranging, and taking natural logarithms

$$\log (d\mu/dt) = 2 \log\mu_t + g + at \tag{5}$$

where g = log ($-a\, b/C$). This suggests setting up a model of the form

$$\log y_t = 2 \log Y_{t-1} + g + at + \varepsilon_t, \qquad t = 2,...,T \tag{6}$$

where $\varepsilon_t \sim N (0, \sigma_\varepsilon^2)$. Parameters a and g are estimated by regressing log ($y_t \mid Y_{t-1}^2$) on time using "Method of least squares". The p - step - ahead forecast of $\hat{y}_{T+p|T}$ can be computed from the recursions

$$\hat{y}_{T+p|T} = \hat{y}_{T+p-1|T} + \hat{y}_{T+p|T},\ p = 1, 2, ... \tag{7}$$

where

$$\hat{y}_{T|T} = y_T$$

3. *Structural time-series model for level (STML) :* Following Harvey[7], STML based on logistic growth curve is

$$\log y_t = \mu_t^* + \varepsilon_t, \tag{8}$$

$$\mu_t^* = \mu_{t-1}^* - a_{t-1} + a^* \exp (\mu_{t-1}^*) + \eta_t, \tag{9}$$

$$a_t = a_{t-1} + \xi_t, \tag{10}$$

where $\mu_t^* = \log \mu_t$ and ε_t, η_t and ξ_t are mutually independent normally distributed white noise disturbances Also, $\varepsilon_t \sim N(0, \sigma_\varepsilon^2)$, $\eta_t \sim N(0, \sigma_\eta^2)$ and $\xi_t \sim N(0, \sigma_\xi^2)$. Etimation of parameters is carried out using STAMP software package, Version 6.0 (Koopman *et al.*[9]) available on the web *http://www.stamp-software.com.*

Goodness of Fit :

After fitting several competing models to data, the next step is to examine their goodness of fit with a view to selecting the best model. Three criteria, viz. Root Mean Square Error (RMSE), Akaike Information Criterion (AIC), and Schwartz-Bayesian Criterion (SBC) are used for the purpose (Shumway and Stoffer).[10] These are respectively computed from the formulae:

$$\text{RMSE} = \left[\sum_{t=1}^{T} (y_t - \hat{y}_t)^2 / T \right]^{1/2}, \text{AIC} = -2 \log_e L + 2n, \text{SBC} = -2 \log_e L + n \log_e T$$

In the above, y_t and $\hat{y}_t$ denote respectively the i^{th} observed and predicted value, T is the total number of observations, L is the likelihood function and n is the number of hyperparameters estimated. Lower the values of these statistics, better is the fitted model.

Results and Discussion

Data on all-India milk production during the period 1981-'98, obtained from the latest issue of "Agricultural Research Data Book" published jointly by I.C.AR. and I.AS.R.I., is utilized for analysis and is reproduced in Table 1 for ready reference. As this data is on annual-basis, the "Seasonal component" is not present. In the first instance, attempts are made to fit logistic growth model given by eq. (1). As the underlying parameters appear in a nonlinear manner, Levenberg-Marquardt iterative estimation procedure available in NLIN option of SAS[5] software package is used.

However, residual analysis reveals that the assumption of independence of error terms is not satisfied at 5% level as the "Run test" statistic comes out as $Z = -2.08$, which lies in the critical region. Subsequently LM, the logistic growth model with AR (1) error term, given by eq. (2), is fitted to the data using PROC ARIMA module available in SAS[5] software package and the results are presented in Table 2(i).

Residual analysis indicates that now the assumption of independence of error terms is not violated. Subsequently, STMF and STML are fitted to the data set using STAMP (Koopman *et al.*[9]) software package and the results are respectively presented in Table 2(ii) and Table 2(iii).

Table 1–All - India milk production (in million tonnes)

Year	Milk Production
1981	31 6
1982	34 3
1983	35 8
1984	38 8
1985	41 5
1986	44 0
1987	46 1
1988	46 7
1989	48 4
1990	51 4
1991	53 9
1992	55.7
1993	58 0
1994	60 6
1995	63 8
1996	66 2
1997	69.1
1998	70 8

To select the best model among the three competing models, viz. LM, STMF and STML for describing the data under consideration, three measures of goodness of fit are computed and the results are exhibited in Table 2 (iv). A perusal shows that the values of all the three measures are least for STML. Hence structural time-series model with level is found to be the best model for describing all-India milk production data under consideration. Finally, its forecasts for the next five years are reported in Table 2(v).

Table 2–Fitting of statistical models to all - India milk production data

Parameter	Estimate
(i) Logistic model with AR (1) *error (LM)*	
a	0.08
b	2 87
C	116 73
AR (1)	0 62
(ii) Structural time-series model for flow (STMF)	
Constant (g)	6 22
a	0 85
(iii) Structural time-series model for level (STML) :	
μ_t	70 80
a_t	2 31
σ_ε^2	0.12
σ_η^2	0.45
σ_ξ^2	0.08

Table 2 Contd..

Table 2 Contd..

(iv) Goodness of fit statistics

	Model		
Statistic	*LM*	*STMF*	*STML*
RMSE	0 67	0.44	0.40
AIC	44 46	37 67	25.97
SSC	46 24	40 34	27.01

(v) Forecast values based on STML (in million tonnes)

1998-99	70 99
1999-00	72 47
2000-01	72.54
2001-02	73 95
2002-03	75 44

In this paper, attempts are made to describe the path of all-India milk production over time using "Structural time-series modelling" approach. Although, explicitly time is the only explanatory variable, yet implicitly each observation is net result of effects of a large number of factors, like species-wise bovine population, feed and fodder availability, and investment on animal husbandry and dairying. Advantage of having one explanatory variable is that it is possible to study deeply the underlying nonlinear functional relationships in an analytic manner. An alternative approach is to consider several explanatory variables at the same time but then disadvantage is that the model has to be restricted to linearity, which may not hold in reality. Or else, study a comprehensive model in which separate equations are developed for each explanatory variable. Although such a modelling approach does provide a lot of useful information, its main criticism is that efficient estimation of the large number of parameters involved is not possible.

Concluding Remarks : Modelling and forecasting all - India milk production is of immense help in arriving at optimum policies concerning various aspects of dairy industry. With international prices of products, like skimmed milk powder going up and favourable WTO regulations on subsidies, Indian dairy producers are probably for

the first time getting the cream from an otherwise washed-out international dairy scene. A country like Holland, which is known to be a dairy country with its cheese and milk products, is importing its requirements from India. In order to take full advantage of this changed scenario, modelling and forecasting all-India milk production precisely has become still more important and, to this end, the methodology described in the present paper would hopefully play an important role.

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Ultrastructural investigation of caudal neurosecretory cells in an Indian catfish, *Clarias batrachus*

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Abstract

Ultrastructure of caudal neurosecretory cells scattered in the posterior end of spinal cord was studied in an Indian catfish *Clarias batrachus* Pattern of secretary activity, along with all the cellular elaboration of these neurosecretory cells was observed and its significance was briefly discussed

(Keywords : caudal neurosecretory cells/Indian catfish/ *Clarias batrachus*)

Introduction

Caudal neurosecretory system well known in fish, has long been of interest to fish biologists, following first observation by Weber[1]. Subsequently morphological components were investigated by Enami[2] and Enami *et. al.*[3] and functional link between the neurosecretory cells and a secretory product storage and release organ - the teleostean urophysis was described[4]. Extensive comparative studies are available for teleosts and elasmobranchs[5-7]. Following these early studies, the caudal neurosecretory system and its main secretory products, the peptides urotension I and urotension II have been described in many species of fish[8,9], the biological action of which reveals its involvement in osmoregulation and reproduction[10-23]. It has recently been shown using homologous radioimmunoassay, that plasma concentration of urotensin II is significantly elevated in sea-water as compared to fresh water adapted fish[24-25]. Furthermore the urophysial content of neurosecretory material becomes depleted in response to hyperosmotic challenge[26].

Immunohistochemical and biochemical studies indicate adrenergic, cholinergic, serotonergic and peptidergic inputs to the brain[27-31]. The action of specific neurotransmitter/modulators on these caudal neurosecretory cells, in an *in vitro* caudal neurosecretory system preparation has been investigated on euryhaline flounder[25,32].

Ultrastructure of caudal neurosecretory cells was initially studied on Japanese eel *Anguilla*[3], later in *Tinca vulgaris*[33] and *Fundulus heteroclitus*[34]. Following these

investigations, many studies have been undertaken to reveal the ultrastructural morphology of the caudal neurosecretory cells which have revealed characteristic of neurosecretory cells in general[17,35,39].

Although a detailed description of morphological characteristic in Indian teleosts is available[40], ultrastructure has received minimal attention. In an attempt to elucidate the ultrastructural organization of the neurosecretory cells of caudal neurosecretory system of an Indian teleost, investigation was carried on in an Indian catfish species *Clarias batrachus* and observations were compared with ultrastructural details observed in other teleosts.

Materials and Methods

The fresh specimen of *Clarias batrachus* were procured from local market and acclimatized in laboratory condition. The posterior end of spinal cord, corresponding to the last 8 to 10 vertebrae, was removed along with urophysis and filum terminale, from anaesthetized fish, after 12 days of acclimatization. The dissected tissue was fixed in 2.5% glutaraldehyde, prepared in 0.1 M caccodylate buffer (pH 7.4) at room temperature. It was postfixed with 2% osmium tetraoxide, to be quickly dehydrated in acetone and embedded in epoxy resin. Semithin sections (1 μm) were then cut in sagittal and longitudinal planes. Section obtained were stained with Toluidene blue, mounted in canada balsom and examined under light microscope to locate the neurosecretory cells. After marking the area in semi-thin sections, fine sections were cut on an ultramicrotome, mounted on grids, stained with uranyl nitrate and lead citrate and examined under electron microscope (Philips).

Observation

Sagittal sections of the caudal neurosecretory system in *Clarias batrachus* clearly demonstrate presence of a central canal (c.c) clustered with ependymal cells (e.c) close to it (Fig. 1 a,b) and scattered neurosecretory cells (Fig. 1 a,d), located ventro-laterally in different profiles. In addition to clustered cells, solitary neurosecretory cells were also observed (Fig. 2 a,b) insinuated with ependymal cells (Fig. 1c).

The unitary cells show a characteristic pear shaped outline in longitudinal plane (Fig. 2 a,b,d) with a prominent spherical nucleus (n), double layered nuclear membrane (nm) and one or more nucleoli (nl). Nucleus is seen with homogeneous chromatin (ch). Nucleus cytoplasmic ratio is varying at different places. Significant feature of the cytoplasm is accumulation of endoplasmic reticulum (ER), mitochondria (m) and golgi bodies (G). Mitochondria are mostly found close to the

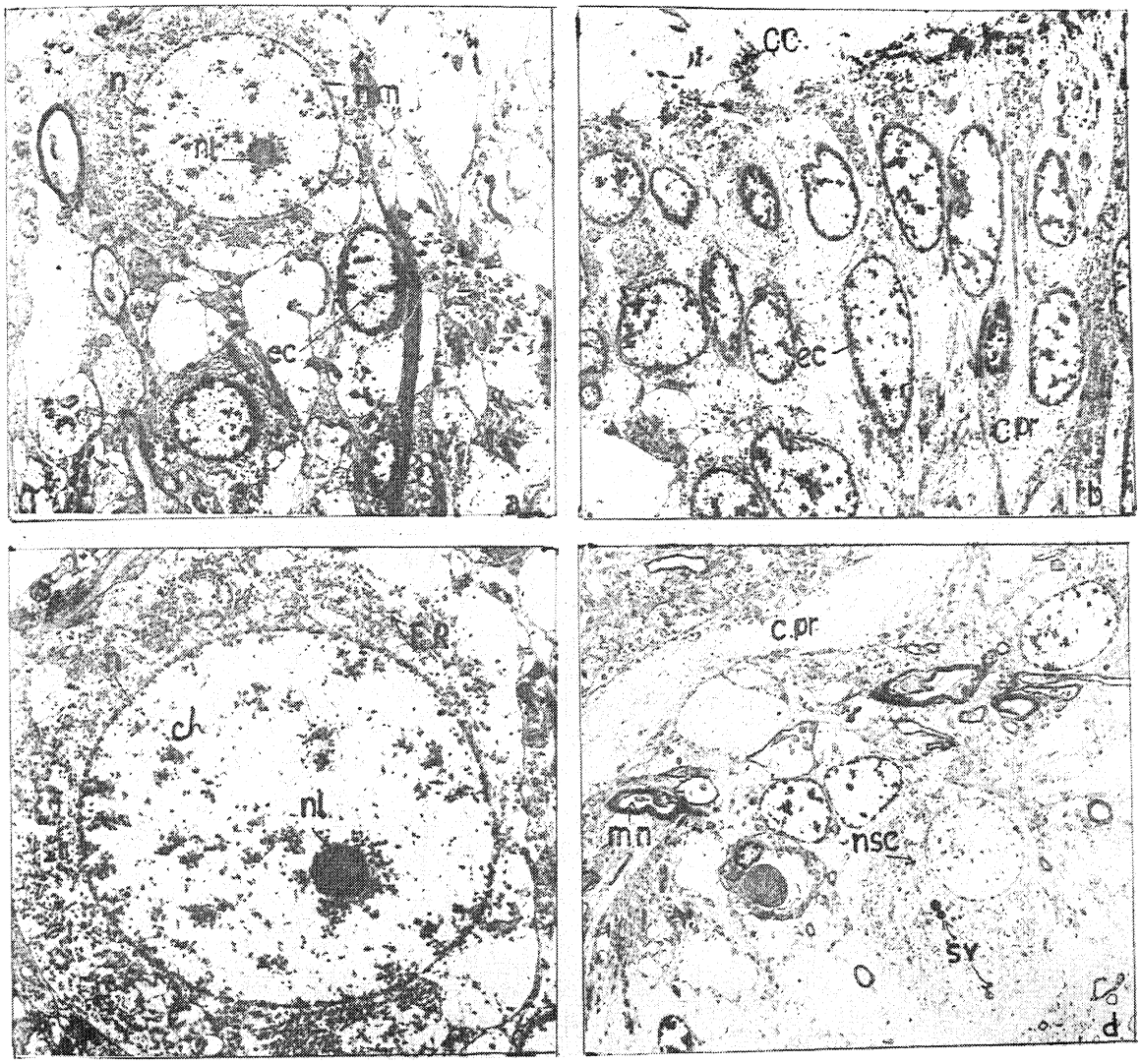

Fig. 1– Electron-micrograph of caudal neurosecretory system of *Clarias batrachus*

(a) showing neurosecretory cell with large, round nucleus (n) and a prominent nucleolus (nl). X 1450. (b) showing celL processes (c.pr) and ependymal cell (e.c) along central canal (cc). X 1150. (c) showing neurosecretory cells, nucleus with few nucleoli. X 1450. (d) showing neurosecretory cells, myelinated nerves, cell processes and small vesicles. X 1450.

c.c : Central canal, ch : Chromatin, c.pr : Cell process bundle, d.cv : Dense core vesicles,
e.c : Ependymal cells, ER : Endoplasmic reticulum, E.sg : Elementary secretory granules,
G : Golgi apparatus, g.dcv : Granular dense core vesicles, m : Mitochondria,
n : Nucleolus, nsc : Neurosecretory cells, nm : Nuclear membrane, nl : Nucleus,
r.ER : Rough endoplasmic reticulum, s.v. : Small vesicles

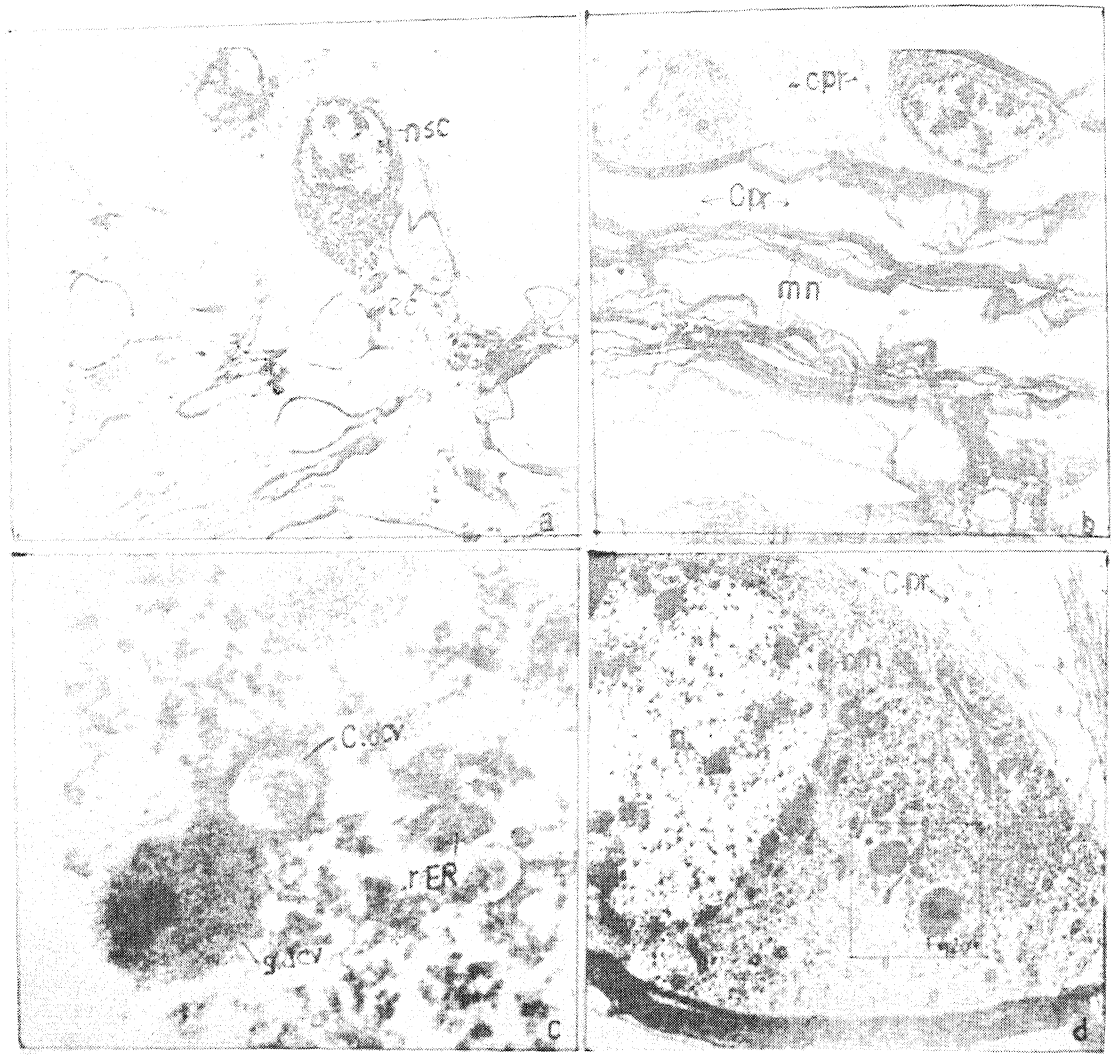

Fig. 2– Electron micrograph of a caudal neurosecretory cell of *C. batrachus*

(a) showing pear shaped outline, nucleus and ependymal cells. X 850. (b) showing myelinated nerve fibre and cell processes. X 850. (c) showing dense core vesicles (d.cv) and rough endoplasmic reticulum (r.ER). X 1450. (d) showing pear shaped outline, nucleus (n), nucleoli (nl) and dense cored vesicles (d.cv). X 4200.

nucleus (Fig. 3 b,c,d). The cytoplasm contains large accumulations of elementary secretory granules (sg), mostly in vicinity of golgi zone (g). The granules are of several size and colour. Some of the dense granules collect together with a dense core and a surrounding membrane (Fig. 2c, 3 a,b). Substance within these vesicles have colloidal to granular appearance. This is characteristic of primary granules which are of the size of approximately 80-200 nm. The small sized vesicles, of about 60 nm, are also encountered with electron dense or electronluscent textures (Fig. 3a) which may be synaptic vesicles containing acetylcholine. Elementary granules of various size and texture, demonstrate different stages of secretion.

The cytoplasm is occupied by the organelles similar to those found in the cytoplasm of a neurosecretory cell. Immediately encircling the nucleus are several profiles of rough endoplasmic reticulum (r.ER) and clusters of free ribosomes. Characteristic of this cytoplasm is accumulation of granules with a dense core and a surrounding limiting membrane with a space in between (Fig. 2c, 3a). Golgi membrane system is not prominent.

The dense core vesicles under TEM confirmed light microscopic observation. Each measures 80-165 nm and the substance therein shows colloidal to granular appearance. Some vesicles are opaque, others exhibit an electron gray content, while the mature ones displays an electron dense core, surrounded by a clear outer rim. However, large accumulation of neurosecretory granules (Herring bodies) and lysosomes were not observed.

The neurosecretory axons terminating on a perivascular basement membrane are rich in connective tissue fibres. This membrane may send deep complex extensions into the termination of neurosecretory axons.

Discussion

The ultrastructural observation of, caudal neurosecretory cells in catfish *Clarias batrachus* revealed clearly all the features of neurosecretory cells that may be involved in producing a peptidergic substance. It clearly confirmed the observation made under light microscope, demonstrating elaborately the necessary organelles for synthesis, transport and release of secretory materials observed in caudal neurosecretory system of teleosts studied[6,17,38,39]. Perikaryal organelles, required for synthesis of a secretory substance, include nucleus, nucleolus, mitochondria, endoplasmic reticulum. Golgi membrane system, free ribosomes, glial cells, which are conspicuous under TEM.

The elementary granules containing little electron dense material or entirely devoid of this material have been observed in *Leuciscus rutilus* and *Phoxinus phoxinus*[36] and *Cyprinus carpio*[37], as well as in marine teleost also[12]. Small vesicles, presumably of synaptic nature, containing acetylcholine, have also been observed in carps and other teleosts[37,41,42]. Morphologically many of these small vesicles seem to arise from the fragmentation of the membranes of empty elementary granules[12,43]. Presence of two type of granules suggest possible presence of at least two different active substance.

The light microscopic observation of the caudal neurosecretory cells is confirmed by TEM in C. *batrachus*. The findings are similar to those of other teleosts[14,44,45]. It has been shown by bioassay and chemical methods that the urophysis contains a urophysial hormones and a high concentration of Ach[41,45]. Two types of secretory granule larger and smaller ones have been observed in all the teleosts observed[34,37,46].

Accurate analysis of the secretory activity of the caudal neurosecretory cells can be made only with an electron microscope, since the dimension of elementary neurosecretory granules of secretory products are below the resolution of light microscope. But interpretation of the secretory activity o neurosecretory cells, based on the result obtained remains uncertain[36]. The ultra structural details of the cell organelles such as rough ER and golgi complex that participate in the formation of secretory material, are elucidated for the first time in an Indian catfish species.

Ultrastructural observation of caudal neurosecretory system of *Clarias batrachus* clearly revealed secretory organization of caudal neurosecretory cells. The caudal neurosecretory neurons demonstrate all the finer elaboration of a neuroendocrine cell in their structure. The cellular details of the neurosecretory cells of caudal neurosecretory system as observed under electron microscope in *Clarias batrachus* are similar to neuroendocrine cells described in other vertebrates.

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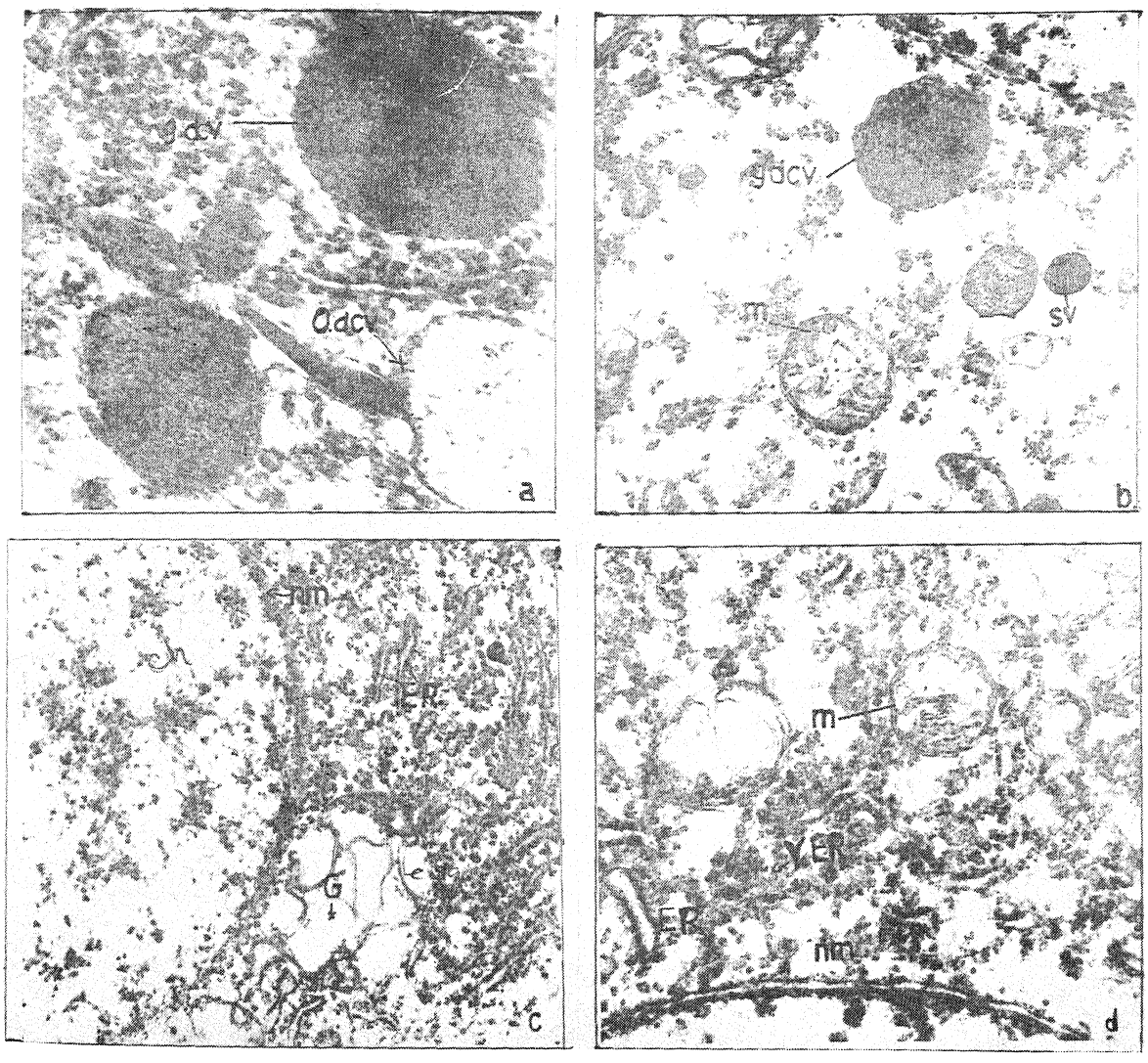

Fig. 3– Electron micrograph of caudal neurosecretory cells of C. *batrachus*

(a) showing magnified view of three types of dense cored vesicles. X 21500. (b) showing granular dense cored vesicles, small vesicles. X 21500. (c) showing nuclear membrane, chromatin, golgi body, endoplasmic reticulum. X 21500. (d) showing mitochondria, nuclear membrane, endoplasmic reticulum (ER). X 21500.

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Bimodal oxygen uptake in relation to body weight and seasonal temperature of freshwater crab *Paratelphusa spinigera*, Wood Mason (Crustacea decapoda)

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Abstract

The freshwater crab *Paratelphusa spinigera* Wood Mason (Crustacea : Decapoda) is structurally and functionally well adapted for aquatic as well as aerial mode of respiration. Oxygen uptake rate during aquatic and aerial respiration of *P spinigera* of different weights have been measured during winter (17 7 ± 1.5 °C) and summer (32.8 ± 0.65 °C). During winter oxygen uptake from air ranged from 0.4 mlO_2h^{-1} to 2 6 mlO_2h^{-1} and from water from 0 349 mlO_2h^{-1} to 2 512 mlO_2h^{-1} respectively with an increase in body weight from 4.5 g to 55.6 g. In summer, aerial and aquatic oxygen uptake ranged from 1 0 mlO_2h^{-1} to 2.7 mlO_2h^{-1} and 0.698 mlO_2h^{-1} to 2 512 mlO_2h^{-1} respectively for crabs ranging from 4.5 g to 45 7 g body weight. With unit increase in body weight, the oxygen uptake in aerial and aquatic respiration during winter increased by powers of 0 72494 for aerial and 0.73698 for aquatic respectively. In summer, they increase by powers of 0.603 and 0.954 for aerial and aquatic oxygen uptake respectively Negative correlation between body weight and metabolic rate was observed. The correlation coefficient between body weight and oxygen uptake in both aerial and aquatic mode of respiration is highly significant ($P < 0.001$).

(Keywords · Freshwater crab/Crustacea/Decapoda/*Paratelphusa spinigera*)

Introduction

The freshwater crab *Paratelphusa spinigera* are abundantly found in/on the mud-soil of the banks of ponds, puddles and lakes/wetland of North Bihar. Of the 4500 species of brachyuran crabs so far known, many of which live in intertidal zones, but can withstand various degrees of exposure to air. Freshwater crabs belong to the family Potamonidae[1] Although *P. spinigera* is basically a freshwater crab, this species is well adapted for terrestrial habitation like most other potamonid crabs[2]. Respiratory

organs consist of 7 pairs of gills and one pair of branchial chamber on either side of the thoracic region. The gill .chamber is enclosed between the branchiostegite or a part of carapace on the outer side and the thoracic wall on the inner side. Inner epithelium of branchiostegite i.e., branchial epithelium becomes highly vascular and is supplied with deoxygenated systemic blood from the thoracic sinus. Morphohistory of respiratory system of freshwater crab *Barytelphusa cunicularis* was described[3]. In aerial respiration an important role is played by thin epithelium that lies beneath the branchiostegites[3,4] lining branchial chamber. The dissolved oxygen as well as temperature play a significant role in the growth of this aquatic organism. Hence it is of interest to evaluate oxygen requirement of *P. spinigera* in different habitats such as aerial and aquatic. The respiratory responses of *Carcinus maenus* to salinity changes, in environment was reported[6]. The influence of thermal acclimatation on oxygen consumption of *Paratelphusa hydrodromus* was studied[7], while the respiratory metabolisms in *Barytelphusa guerini* in relation to body size, sex and gill area was also reported[8]. The oxygen requirement in edible mud crab, *Scylla serrata* during its molt cycle in different salinity conditions has also been established[9]. The present paper is an attempt to evaluate the effects of body weight and seasonal temperatures on the bimodal gas exchange in *P. spinigera.*

Materials and Methods

Live specimens of *Paratelphusa spinigera* of different weight groups were brought from Kawar lake, Begusarai (Bihar). The study was carried out in Freshwater Laboratory, P.G. Department of Zoology, T.N.B. College, between July and August 2000 (32.8 ± 0.65 °C) and between January and February 2001 (17.7 ± 1.5 °C). These crabs were kept in large aquaria having shallow water in one half, and heap of sand on the other half. The crabs were fed regularly with . chopped small fishes. The crabs were acclimatized to laboratory conditions for two weeks.

The aquatic oxygen uptake was measured using a cylindrical glass respirometer as designed[10]. The respirometer was connected to a large constant level water reservoir kept at a height to maintain water flow. Clamps were used to make adjustment, thus making the water flow constant. A single, specimen was weighed and placed in the respirometer and then the respirometer was completely filled with reservoir water. Clamps at the inlet and outlet of cylindrical glass respirometer were adjusted to stabilize the flow rate of water depending on the size of the crab, so that the crab did not feel uneasy within the chamber and breathe normally. The respirometer was covered with a black cloth, so that the crab may not be disturbed. After weighing the

crab, it was left to acclimatize in glass respirometer for at least one hour and then the flow rate was finally checked. Dissolved oxygen content of inspired water was measured. Samples of expired water were taken on every half an hour for two hours from the flask at the outlet of respirometer. Dissolved oxygen content was estimated by Winkler's Method[11].

The difference in the content of dissolved oxygen of the inspired and expired water, together with the rate of flow was used to calculate the oxygen uptake by the respective crab. Weight of crab was applied to estimate the rate of oxygen uptake per unit body weight.

Aerial oxygen uptake of individual crab was measured by using closed glass respirometer of 2L capacity. The crab was weighed/and kept in it. The top of the respirometer was connected to a manometer by means of a latex tube and dipped in measuring cylinder containing kerosene oil. After weighing, the individual crab was placed in respirometer. The respirometer was sealed after 30 minutes at a constant temperature water bath and manometer readings were taken after every half an hour, for two hours. Pellets of Potassium hydroxide (KOH) were kept in a small petridish hanged under the rubber cork to absorb the carbondioxide liberated during the respiration. The aerial oxygen uptake was measured by determining the amount of oxygen needed to neutralize the imbalance of the fluid in the manometer.

Results and Discussion

During winter season (17.7 ± 1.5 °C) the oxygen uptake through the air breathing organs and the gills gradually increased from 0.400 $ml O_2 h^{-1}$ to 2.600 $ml O_2 h^{-1}$ and 0.349 $ml O_2 h^{-1}$ to 2.512 $ml O_2 h^{-1}$ respectively with increase in body weight from 4.5 g to 55.6 gm (Table 1). When the estimated data on aerial and aquatic oxygen uptake were plotted against body weight on log/log co-ordinates, they gave straight lines with slopes of 0.725 and 0.736 respectively with an intercept of 0.11796 and 0.09835. The results show a very high degree of correlation for aerial ($r=0.8873$; $p < 0.001$) and aquatic ($r=0.80642$; $p < 0.001$) oxygen uptake with body weight. In summer (32.8 ± 0.65 °C) with an increase in body weight from 4.5g to 45.7g the rate of oxygen uptake from air and water increase from 1.00 $ml O_2 h^{-1}$ to 2.70 $ml O_2 h^{-1}$ and 0.698 $ml O_2 h^{-1}$ to 2.512 $ml O_2 h^{-1}$ respectively.

Table 1– Mean values of oxygen uptake VO_2 ($ml O_2 h^{-1}$ $crab^{-1}$) from air & water for different weights of *Paratelphusa spinigera* during winter (17 7 ± 1 5 °C) & summer (32.8 ± 0.65 °C) of Kawar lake (Begusarai), North Bihar

Summer (32 8 ± 0.65 °C)			Winter (17 7 ± 1.5 °C)		
Mean body Wt (g)	Aerial O_2 Uptake $ml O_2 h^{-1}$	Aquatic O_2 Uptake $ml O_2 h^{-1}$	Mean body Wt (g)	Aerial O_2 Uptake $ml O_2 h^{-1}$	Aquatic O_2 Uptake $ml O_2 h^{-1}$
4 532	1 000	0 698	4 500	0 400	0 349
8 372	1 200	0 838	7 500	0.500	0 558
8 800	1 250	1.111	10 000	0.800	0.628
13.565	1 300	1.117	15 000	0 900	0 625
16.800	1 433	1 117	20.000	0.900	0 834
29.000	1 800	1 541	22 000	0 500	0 295
30 650	2 000	1 954	25 000	1 100	1 047
31 750	2 200	2 345	30 250	1.500	1 256
35 750	2 400	2 395	35 000	1 800	1.675
45 750	2 700	2.512	45 000	2 300	2.198
			55 605	2 600	2 512

The intercept values from these parameters were found to be 0.2 and 0.0632. The log/log plots of the rates of oxygen uptake to body weight gave straight lines. It was found that with unit increase in body weight of *P. spinigera,* the rate of O_2 uptake from air and water increased by the powers of 0.603 and 0.954 respectively (Fig. 1). The result showed a high degree of positive correlation for aerial ($r = 0.7508$, $p < 0.01$) and aquatic ($r = 0.6788$, $p < 0.01$) oxygen uptake with an increase in body weight (Table 2). Present observation confirmed that this species. can also successfully breathe in terrestrial medium. The branchial epithelium can efficiently extract more oxygen from air than water. It has well suited respiratory organs for aerial respiration. It has also been reported that P. *spinigera* is structurally well adapted for aerial oxygen uptake[2]. He reported presence of swelling tips on the gill lamellae and presence of interlamellar septum, which, according to him, are amphibious adaptations of *P. spinigera* as that of *Barytelphusa cunicularis*[3] and *Barytelphusa guerini*[8]. It has been reported[12] that in the crabs belonging to the family

Pseudothelphusidae aerial exchange is facilitated by extensive modification of the highly vasuclarized branchial epithelium which lies under the branchiostegite. In *Paratelphusa spinigera* also, such highly vascularized branchial epithelium is present. It was also been reported[13] that oxygen uptake of *Pseudothelphusa garmani garmani* in the air at 25 °C scales to the power 0.70, which is slightly higher to the present observation in the air (b=0.603 at 32 °C) in *P. spinigera.* It has been reported[2] that although 7 pairs of gills are primary respiratory organs in *P. spinigera,* the total surface area of gills are in between the purely aquatic crabs and air-breathing crabs. In *P. spinigera* (100g) total surface area of the gill is 40354 mm^2 and for air-breathing crabs, the total surface area of the gill was 5322.20 mm^2 in *G. grayi*[14]. At the same time the surface area of gills of *P. spinigera* (50g) is 27336.70 mm^2 as compared to 38115.97 mm^2 in the aquatic crab[15,16].

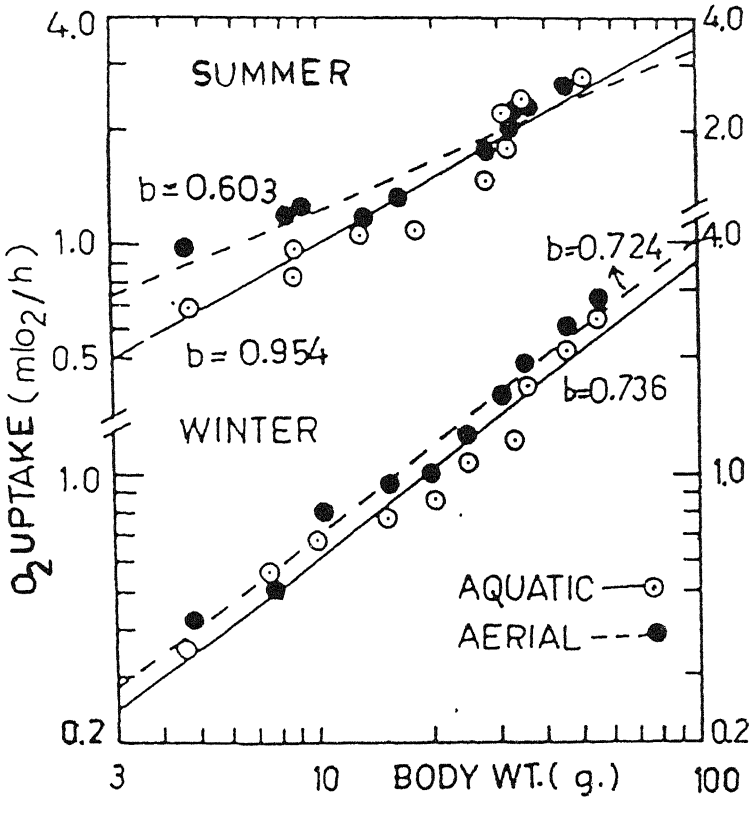

Fig. 1–Log-log graph showing rate of aerial and aquatic O_2 consumption (mlO_2/h) in relation to their body weight in summer (32.8 °C) and winter (17.7 °C) season.

Table 2– Summary of the relationship between oxygen uptake (mlO_2h^{-1} $crab^{-1}$) from still water by the gills and from air by the air breathing organs of *Paratelphusa spinigera* to their absolute body weight

Experimental conditions	Ambient water temperature	Organ for oxygen uptake	Intercept (a) estimated value	Regression coefficient (b) Estimated value	Correlation coefficient
Aquatic medium	Winter	7 pairs gills	0 09835	0.73698	0 80642
	17 7 ± 1 5 °C				p < 0.001
Aerial medium	Do	Brachial epithelium	0 11796	0 72494	0.88730 p < 0 001
Aquatic medium	32 8 ± 0 65 °C	Gills	0.06320	0 95400	0.6788 p<0.01
Aerial medium	32 8 ± 0 65 °C	Brachial epithelium	0.20000	0 06030	0.7508 p < 0 01

(a = Intercept, i e , value for 1 g fish, b = Regression and r = Correlation Coefficient)

In morphological study of crabs from several families, concluded that the branchiostegites were supplied with deoxygenated systemic blood from the thoracic sinuses and returned oxygenated blood to the pericardium. Bimodally breathing crab possesses dual routes for the return of venous blood to the heart. Thus, deoxygenated haemolymph within the thoracic sinus system may return either via the gills as in primarily aquatic crabs or via vascular beds within the branchiostegites. In this way P. *spinigera* can survive totally on terrestrial medium for about seven days. It has been suggested[17,12,18], that branchial epithelium functions like lungs, in crustaceans.

The higher respiratory rate of *P. spinigera* could be correlated to the fact that during summer the rate of O_2 uptake (mlO_2h^{-1}) is significantly higher than winter season, because during summer these are metabolically more active than in winter. The scanty occurrence of these crabs during winter in the month of December and January could be due to their winter sleep or hibernation, when they remain dormant in their burrow. Similar type of winter sleep was recorded by Barnes[1].

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Impact of temperature acclimation on transpiration of the male desert woodlouse *Hemilepistus reaumuri* (Isopoda, Oniscidea) in Benghazi, Libya

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Abstract

The transpiration rates of male desert woodlouse *Hemilepistus reaumuri* (Audouin & Savigny, 1826) acclimatized for 48 hours at temperatures 10,15,20,25,30,33 and 36 °C showed a gradual increase from the lower to the higher temperatures. Significant differences existed between the transpiration rates of these woodlice and temperature acclimations ($F = 18.27$; $P < 0.01$) and also on the transpiration rates between *H reaumuri* acclimatized to different temperatures ($F = 4.31$, $P < 0.05$) However, differences in transpiration rates of these isopods within a particular acclimatized temperature were insignificant ($F = 1.41$, $P > 0.05$). A strong relationship ($\omega^2 = 0.57$) existed between transpiration rates and temperature acclimations of *H reaumuri* Transpiration rates of these woodlice acclimatized at 36 °C were significantly higer when compared with the values of the same recorded at lower temperatures.

(**Keywords**. *Hemilepistus reaumuri*/temperature acclimations/woodlouse/transpiration rates/ Benghazi).

Introduction

Hemilepistus reaumuri (Audouin & Savigny, 1826), the desert isopod, form large proportions of the woodlice in the neighborhood of Benghazi, Libya, are well adapted to life in arid and semi-arid soils. It belongs to the order Isopoda, superfamily Oniscidea and family Porcellionidae[1]. *H. reaumuri* are usually seen active in Benghazi on the surface of hard soils from January/February to May where they burrow the cover of bushes[2]. This species is widespread in North Africa and surrounding regions. Our previous studies[2,3] showed that the water - loss from the body surface of this woodlouse gradually increases with rise in temperature, and acclimatization to 10 or 30 °C for a week has marked effects on its behavior and activity when later exposed to

rising temperatures. The present study investigated the impacts of different temperature acclimatization of the same *H. reamuri* on its transpiration rates, and the strength of association and relationship between these factors.

Materials and Methods

Eight adult male *H reaumuri* (body mass 390 ± 20 mg) were selected from the stock. They were maintained in the laboratory for a week (Temp. 25 ± 1 °C; R.H. 65 ± 2%). Glass vessels (5 cm diameter, 2.5 cm height), containing moist filter papers at the bottom to keep the humidity inside the vessels around 65%, were selected and kept in the Gallenkamp incubator at 10 °C. Dry leaves of *Citrus Umonia* soaked in water to become soft and palatable to these woodlice were placed in the vessels, which served as food for them. Each *H. reaumuri,* after taking its initial body weight, was kept in the vessel and was allowed to acclimatize at 10 °C for 48 hours. After that, the transpiration rate of each animal was measured separately at the same temperature at which it was acclimatized previously. This procedure continued at 15,20,25,30,33 and 36 °C. The highest acclimation temperature was limited to 36 °C, since our previous trial experiments revealed some mortality of these woodlice after 6 to 8 hours of exposure at temperatures 38 °C and above. A wet cotton plug was kept above the filter papers in dishes kept from 30 to 36 °C to keep the filter papers moist and to prevent the *C. limonia* leaf from drying up.

The procedure followed by various workers[3,4,5] to measure the transpiration rates of different species of woodlice were adopted in the present study. Each *H. reaumuri* after the acclimation period of 48 hours at a particular temperature was weighed individually to 0.005 mg and then exposed separately, at the same temperature at which it was acclimated previously, for one hour over phosphorus pentoxide (P_2O_5) before re-weighing. The changes in the body weights of woodlice through transpiration are more pronounced when exposed over P_2O_5 as compared with another desiccant such as Calcium chloride[4]. The duration of one hour exposure in the present study was based on initial trial experiments where these woodlice could withstand this exposure period over P_2O_5 even at high temperatures without showing symptoms of stress or mortality. The results of the transpiration are expressed as mg. cm^{-2} h^{-1}, the surface area of the animal being calculated from the formula $S= kW^{2/3}$, where 'S' is the surface area, 'W' is the initial weight of the animal, and 'k' is a constant. A value of k = 12 was adopted as used for African woodlice[2,3,4,6] and for Indian species[5]. This value is also the mean calculated for British species[7]. The experiment was repeated and there were very little variations between the values of replication tests, and the data were pooled and the mean values taken.

Results and Discussion

The transpiration rates of individual *H. reaumuri* at different acclimated temperatures from 10 to 36 °C are presented in Table 1. There was a gradual increase in transpiration rates of these woodlice from 10 to 36 °C. Repeated measure ANOVA[8] between transpiration rates of these woodlice and temperature acclimations revealed a significant difference ($F = 18.27$; $P < 0.01$). Also, the transpiration rates between *H. reaumuri* exposed to different temperatures were significantly different ($F = 4.31$; $P < 0.05$). However, the differences in transpiration rates of these woodlice within a particular temperature was insignificant ($F = 1.41$; $P > 0.05$) (Table 2). The strength of relationship between the independent variable temperature and the dependent variable transpiration rate was found to be strong, as was evident from Omega-squared (ω^2) value, which was 0.57.

Table 1– *Hemilepistus reaumuri* Transpiration (mg cm^{-2} h^{-1}) of each of eight woodlice kept at different temperatures (10 to 36 °C) for one hour, after acclimatizing them for 48 hours at temperatures 10 °C to 36 °C, keeping the relative humidity 65 ± 2%

Animal numbers	Transpiration (mg $cm^{-2}h^{-1}$)							
	Temperature (°C) exposed							
	10	15	20	25	30	33	36	ΣY
1	1.598	1.502	1.495	1.790	1 835	2 502	2 582	13 304
2	1.333	2.219	1 501	2.212	2 415	2 769	3.004	15 453
3	0.198	1.193	2.399	2 487	1.814	1 585	2.008	11.684
4	0 891	1.156	1.782	2 371	2.646	2.685	2 895	14 426
5	0.749	1.072	1.534	1.746	1.539	2 400	2 885	11 925
6	0 420	0.796	1.309	1 322	1 580	1 261	1.977	8 665
7	1.395	1.682	1 559	1 415	2.484	2 041	3.395	13.971
8	1.001	1.581	1.369	2.138	2.232	2 508	3.168	13 997
ΣX	7 585	11.201	12.948	15.481	16.545	17 751	21 914	103.425
X	0.948	1.400	1.619	1 935	2 068	2 219	2.739	

Table 2–Repeated Measure ANOVA Table

Source	SS	df	MS	F	P
Transpiration between temperatures	16.41	6	2.74	18 27	< 0 01
Transpiration within temperatures	10 40	49	0 21	1 41	> 0 05
Transpiration between woodlice	4.53	7	0 65	4.31	< 0.05
Error	5 87	38	0.15		
Total	26 81	55			

Table 3 shows the *t*-values of transpiration of *H. reaumuri* between two acclimatized temperatures. A significant difference in transpiration rate was discernible between woodlice acclimatized between 10 °C and 20 °C to 36 °C. A similar trend was discernible between the transpiration values at 15 °C and 25 °C to 36 °C. However, the differences in transpiration rate of *H. reaumuri* acclimatized at 20 °C were significantly different with those of 33 and 36 °C, and the same at 25,30 and 33 °C with those of 36 °C only.

Table 3–*Hemilepistus reaumuri* : Fisher's LSD Table showing the t-values of transpiration between two acclimatized temperatures

Temperature	10 & 15	10 & 20	10 & 25	10 &30	10 & 33	10 & 36
t-value	2.30	3.41*	5 02*	5 70*	6.47*	9 11 *
Temperature		15 & 20	15 & 25	15 & 30	15 &33	15 &36
t-value		1 11	2.72*	3 40*	4.17*	6 81 *
Temperature			20 & 25	20 & 30	20 & 33	20 & 36
t-value			1.61	2.28	3 05*	5.70*
Temperature				25 &30	25 & 33	25 & 36
t-value				0.68	1 45	4.09*
Temperature					30 & 33	30 & 36
t-value					0.77	3.41*
Temperature						33 & 36
t-value						2.65*

*Significantly different at 5% level

From our previous study on *H. reaumuri,* we could tabulate the exponential regression model[3] showing the relationships between temperature and transpiration rate in non-acclimatized *H. reaumuri* as $y = e^{-2,175+0\ O98x}$, $R^2 = 98.22$; $F = 4298.21$; $P = 0.000$, where y = transpiration rate, x = temperature, R^2=multiple correlation co-efficient, $F = F$-ratio, P = Probability. Also, the least square fits and their constants[9] ($y=aT^2 + bT +c$; where y = Transpiration rate, T=Temperature, a,b,c= Constants) from the experimental values of temperature-transpiration rate of non-acclimatized *H. reaumuri* was tabulated as $y=0.008960\ T^2 -0.277200\ T +2.440010$. While comparing the transpiration rates of non-acclimatized *H. reaumuri*[3] at different temperatures from 10 to 40 °C with the values of the present study, it was observed that the transpiration rates of the acclimatized *H. reaumuri* from temperatures 10,15,20 and 25 °C were higher than the values recorded for non-acclimatized ones. This trend changed from temperature 30 °C onwards, where the transpiration rates of the acclimatized woodlice were less than those recorded for non-acclimatized ones.

The cuticle of land isopod is more permeable than that of insect[10], and it was previously believed that a water-proofing mechanism, an oriented layer of lipid molecule, is lacking in isopods[11]. Later, however, evidence suggested that some type of water-proofing mechanism may be present in some species[12,13]. Transpiration rates of several species of woodlice at different temperatures demonstrate a sharp increase in higher temperatures[2,3,4,5,6,12,14,15]. This suggests that the water-proofing barrier ability is reduced at higher temperatures. This might be the reason why acclimatization of *H. reaumuri* at 40 °C failed, and a very sharp increase in transpiration rate of the non-acclimatized woodlouse was observed[3] at this temperature (6.50 mg/cm^2/h)[3] . However, the lower transpiration rates of acclimatized *H. reaumuri* at higher temperatures from 30 °C onwards suggest the impact of acclimatization on the water-proofing barrier of this woodlouse to keep the transpiration rate at a lower level when compared with the values of non-acclimatized ones.

In conclusion, temperature acclimations have a profound impact on the transpiration rates of *H. reaumuri.* In the wild, the temperature ranges of the habitats of these woodlice during their maximum surface activity period from January/February to May were well within their tolerance limits. Although its physiology is remarkably well adapted to desert life [16,17], *H.reaumuri* would unlikely to survive a single summer day without the protection of its burrow[18]. Thus, the favorable soil and climatic conditions coupled with an abundant supply of foliage helped these animals to colonize in large numbers in Benghazi, "as they have elsewhere in Mediterranean coastal region.

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Induced autotetraploidy in coriander (*Coriandrum sativum* L.)

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Abstract

An autotetraploid was induced in *Coriandrum sativum* L. cv NP(D)95 following 3 hours treatment of seedlings for 2 consecutive days with 0 25% colchicine Three tetraploids raised at C_1 generation (C_{1-1}, C_{1-2} and C_{1-3}) from the seeds of C_0 plant were compared to normal diploids The tetraploids had 2n = 44 chromosomes, and at meiosis they mostly formed quadrivalents (0 to 6), bivalents (3 to 18) and univalents (0 to 36) in varying frequencies;' while, the control diploid plants (2n = 22) produced mainly bivalents (10.93/cell) and few univalents (0.12/cell). Normal diploid plants had 85.5% pollen fertility and a set of 62.4 ± 2 0 seeds/plant. On the other hand, whereas pollen fertility in the autotetraploids markedly reduced (11.27% to 19 07%), seed set was good (C_0-34, C_1-57, C_{1-2}-27 and C_{1-3}-30). The fruits of C_{1-1} were histologically examined and it was noted that the autotetraploid had more vittae of larger size as compared to the diploid

(**Keywords**: coriander/colchicine/autotetraploids/seed set/vittae)

Introduction

Colchiploidization offers a scope for the development of novel and superior plant types through selection from widened amplitude of variation in the gene pool and it has been successfully utilized in different crop plants for improving various traits[1-5] Although coriander *(Coriandrum sativum* L., family-Umbelliferae) is a major spice of commerce[6] (both leaves and seeds are used as spices), reports on the induction of autotetraploids in this species are rather meagre[7-8] and the tetraploids induced earlier were of non-productive types yielding a few abortive seeds. This paper describes some of the seed fertile autotetraploids in *C. sativum* raised from colchicine treatment.

Materials and Methods

Colchiploidization was attempted on a cv. NP(D)95 of *Coriandrum sativum* L., a variety released by ICAR and cultivated in different states of India, including W.

Bengal[9]. Meristematic tips of young seedlings bearing only two cotyledonary leaves were given a 3 hour treatment for one day or two/three consecutive days with either 0.25% or 0.5% colchicine (Table 1). From a lot of 60 treated plants, one autotetraploid could be raised at C_0 (Table 1) and 20 seeds sown from Co plant produced 3 autotetraploids at C_1 (C_{1-1},C_{1-2} and C_{1-3}).

Table 1–Number of tetraploid plants induced from colchicine treated *C. sativum* seedlings

Colchicine concentration (%)	Days to treatment	No of treated seedlings	No. of autetraploid obtained
0 25	1	10	
0 25	2	10	01
0 25	3	10	.
0 50	1	10	...
0 50	2	10	.
0 50	3	10	...

Morphological, cytological (PMC squashes performed from microsporophyll stained in 1 % propionocarmine solution) and pollen studies were made in the autotetraploids and compared to normal diploids raised under similar conditions. Fully stained pollen grains in 1 % propionocarmine solution were considered fertile. Chlorophyll pigment was estimated from leaf tissues following the method of Arnon[10]. Mature fruits (=seeds) of C_{1-1} and control were histologically examined to assess the number and size of vittae (mericarp possesses essential oil). Microtome preparations were made after fixation of fruits of identical maturity in acetic-alcohol (1:3 v/v), dehydration, embedding and sectioning (15μ) by usual method of Johansen[11]. The sections were stained with safranine. Viability of seeds was studied in C_{1-1} and in control plants following the use of 1 % aqueous solution of tetrazolium chloride for 12 hours and the embryo of half seeds stained red were considered viable.

Results and Discussion

Morphological comparisons :

As compared to normal diploids, the induced autotetraploid plants of Co and C_1 generations showed stunted growth, reduced number of branches and decompound leaves, thick broad dark green pinnae with enhanced chlorophyll (mg/gm of tissue)

content, reduced number of stomata with larger guard cells, delayed flowering with reduced number of compound umbels and umbellets, and increase in pollen size (Table 2).

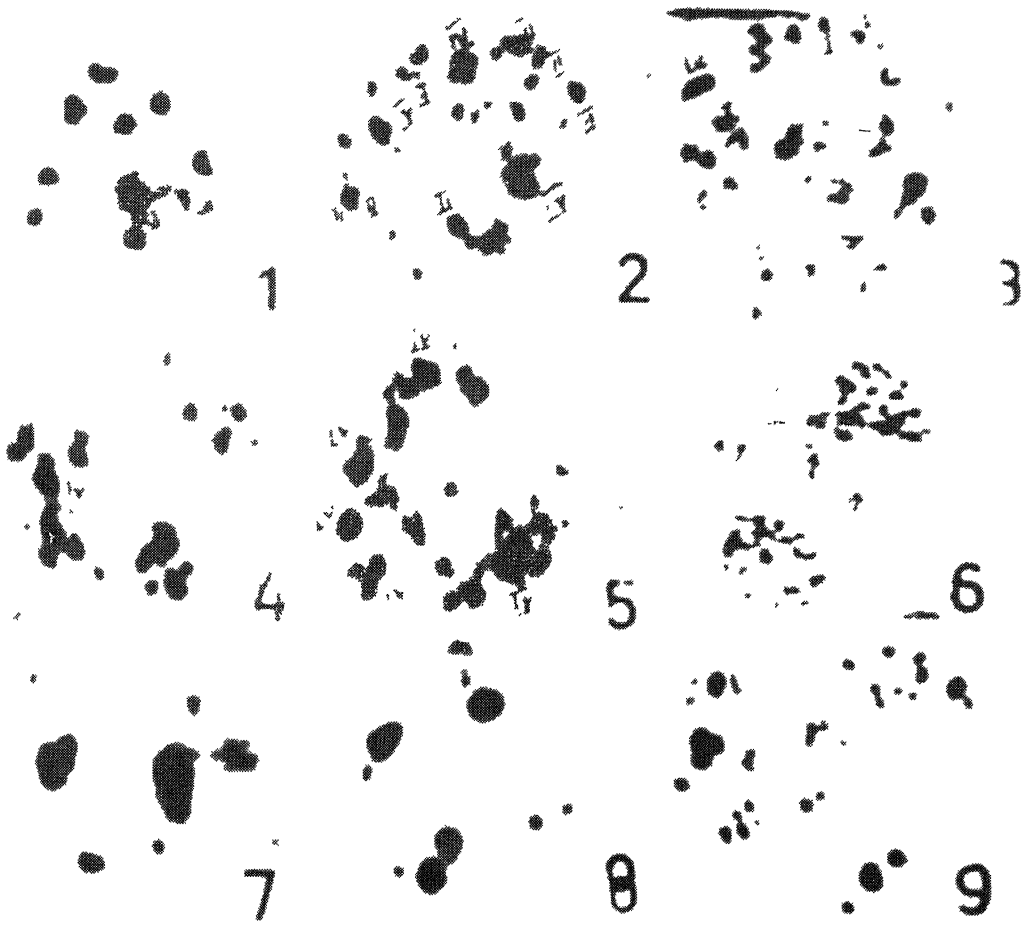
Table 2–Morphological attributes in normal diploids and in autotetraploids of *C sativum.*

Attributes	Normal diploids	Autotetraploids
Plant height (cm)	3.8 ± 2 6	25.0 –31.2
No of primary branches/plant	2.0 ± 1 4	1 0
No of total branches/plant	3 7 ± 2.1	2 0 – 3.0
No. of decompound leaves/plant	21 6 ± 2.3	6.0 – 12.0
Area of pinnae (sq cm) Chlorophyll content of pinnae (mg/gm of tissue)	4.4 ± 0 7	6.8 ± 0.8 – 7. 7.1 ± 0.8
Chlorophyll-a :	0.95	1.36 – 1.40
Chlorophyll-b ·	0.60	0.79 – 0.81
No. of stomata/field (212457.14 sq m)	10.6 ± 1.6	8.5 ± 1.4 – 8.6 ± 1.4
Length of guard cells(μ)	47 2 ± 0.2	69 5 ± 0.2 – 71.3 0 2
Breadth of guard cells(μ)	28.2 ± 0 1	35 02 – 35.7 ± 0.2
Days to first flowering (days)	49-55	67
No. of compound umbel/plant	3.4 ± 1.9	2.0 – 3 0
No. of umbellets/plant	9.6 ± 0.9	3.0 – 5.0
No. of seeds/plant	62 4 ± 2.0	30.0 – 57.0
Pollen fertility (%)	85.5	11.3 – 19.1
Length of pollen grains (μ)	47.0 ± 0.2	59.0 ± 0.2 – 61.0 ± 0.2
Breadth of pollen grains (μ)	25.0 μ 0.2	31.0 ± 0.2–32.0 ± 0.2

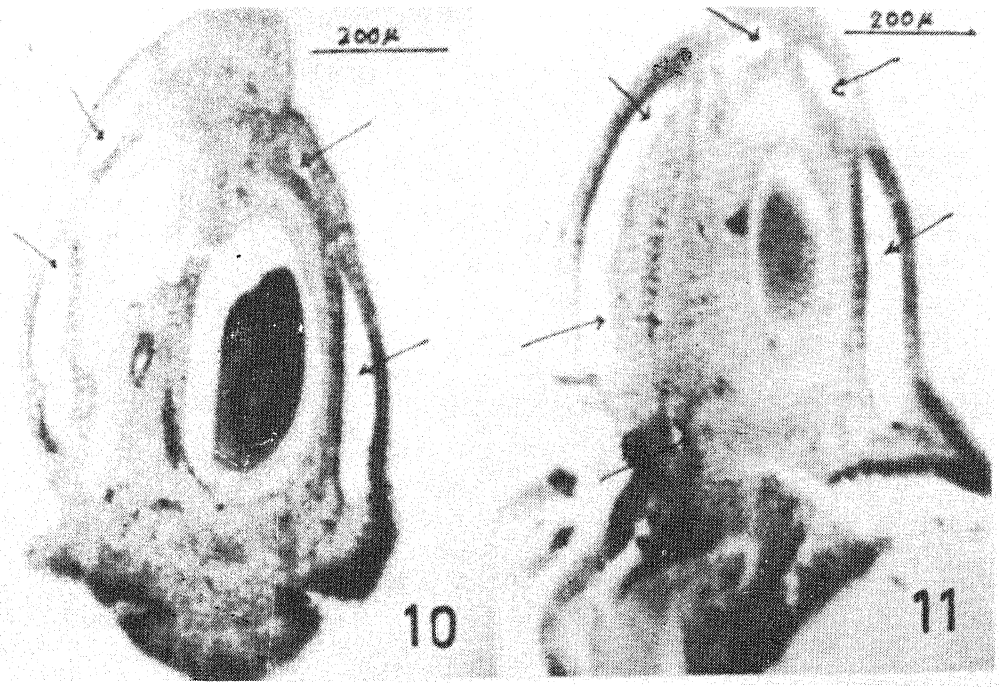
Chromosome behaviour :

Meiotic analysis in autotetraploids is an important part of the study of chromosome behaviour. The parental diploids (Fig. 1) showed essentially bivalents (n= 11), whereas the autotetraploids formed quadrivalents (0 to 6), bivalents (3 to 18) and univalents (0 to 36) in varying frequencies (Figs. 2-5) and their mean per cell values are summarized in Table 3. Trivalent (0 to 1) was only noted in C_0 (Table 3). Co-efficient of qudrivalent realization was 0.428 in C_0, 0.359 in C_{1-1}, 0.411 in C_{1-2} and 0.389 in C_{1-3}. About one-third to two-fifth of the potential quardrivalents were realized in the tetraploids. Frequency of univalent and bivalent per cell was non-random among the tetraploids ($p < 0.001$) but the quardrivalents showed random distribution ($p > 0.70$) as evident from χ^2 test of heterogeneity, thereby indicating constancy of quadrivalent frequency per cell over two generations. Chiasma per cell was 13 ± 0.1 in normal diploids and it varied between 19.3 ± 1.9 and 21.6 ± 1.6 among tetraploids (Table 3). Laggards and multiple groups of chromosomes (Figs. 6-8) appearing in ana-telophase I cells of the tetraploids (23.3% to 40.0%) possibly resulted in the formation of unequal and polysporic (Fig. 9) condition at ana-telophase II. Results indicated that decrease in univalent frequency per cell with concomitant increase in bivalent frequency have reduced anaphasic abnormalities and increased pollen fertility (Table 3), which has also been evidenced from correlation studies made between univalent frequency per cell and abnormal AI cells ($r = 0.97$, $p < 0.01$) and between abnormal AI cells and pollen fertility ($r = -0.94$, $p < 0.05$) of the autotetraploids. Although, pollen fertility was markedly reduced in the autotetraploids they formed good seeds which are neither brittle nor abortive in nature. Furthermore, correlation between pollen fertility and seed yield was nonsignificant ($r=0.17$). Thus, chromosome behaviour in autotetraploids revealed that pollen fertility may be the outcome of chromosomal disturbances arising from pairing irregularities, while seed formation possibly have a genetical basis rather than cytological.

In comparison to normal diploids (seed size: 3.63 mm ± 0.1 X 2.09 mm ± 0.01; seed viability : 88.01 %), seeds of C_{1-1} autotetraploid (seed size: 3.44 mm ± 0.8 X 2.37 mm ± 0.07; seed viability : 60.0%) were only histologically examined as the plant yielded higher number of seeds than other tetraploids (Table 3) and those seeds possessed significantly increased number of vittae (2n : 1 to 4, mean 3.1 ± 0.4, 4n : 1 to 7, mean 4.7 ± 0.8; t value 2.6 for 18 D.F., p value < 0.05) and the vittae (Figs. 10-11) were larger in sizes (2n : 265.48μ ± 1.7 x 69.52μ ± 1.5, 4n: 344.67μ ± 2.4 x 76.90μ ± 1.1). Thus, C_{1-1} offer scope of positive selection among the induced autotetraploids in subsequent generations.



Figs. 1-9–Chromosome configuration in normal diploids (Fig I) and in autotetraploids (Figs 2-9) of *C. sativum* 1-11 II at diplotene (x2000), 2-3 IV + 7 II + 18 I at MI (x 2000); 3 -IV + 18 II + 4 I at MI (x2000), 4-1 IV + 14 II + 12 1 at MI (x 24,00), 5-5 IV + 3 II + 18 I at MI (2400), 6-anaphase I showing laggards (→) and demonstrating irregular separation of chromosomes (x1600), 7 and 8 unequal groups of chromosomes and polysporic condition at A II (x2000).



Figures 10-11–Histological sections of fruits (Fig. 10-2n; Fig. 11-4n) showing vittae (→)

Table 3–Chromosome configurations, pollen fertility and seed set in control (2n) and in autotetraploids of *C. sativum*

	Mean/cell at metaphase I				Chiasma/ nucleus	No. of MI cells scored	Abnormal AI cells (%)	No. of AI cells studied	No of pollen studied	Pollen fertility (%)	Seed set/plant
	I	II	III	IV							
Control (2n) Autotetraploid	0.12	10.93	0.0	0.0	13.6 ± 0.1	110	0.0	51	173	85.5	62.4 ± 2.0
C_0	14.08	8.00	0.08	3.42	20.3 ± :l.3	48	35.3	34	907	14.6	34 0
C_{1-1}	8 31	12.20	0.0	2.87	21.6 ± 1.6	60	23.3	48	215	19.1	57.0
C_{1-2}	14.94	7.94	0.0	3.29	19.3 ± 1.9	34	40.0	60	275	11.3	27 0
C_{1-3}	16.32	7.63	0.0	3.11	20.5 ± 1.9	38	38.5	52	173	14.5	30 0

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Foliicolous lichens and their diversity in north-east India

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Abstract

Lichens strictly colonizing on living leaves are referred here as foliicolous lichens, which are discussed in the present paper North-East India comprising of Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura supports rich foliicolous lichen flora both in luxuriance and species diversity The variable climatic conditions, altitude and high humidity coupled with diverse forest ecosystems are the main factors responsible for rich foliicolous lichen diversity in the region The present study enumerates 74 species of foliicolous lichens distributed in 29 genera and 13 families and forms about 67% of the total foliicolous lichen flora of India In terms of number of species, the genera *Porina* (15 spp), *Strigula* (9 spp) and *Mazosia* (6 spp) show the highest species diversity The study records 3 species viz *Loflammia intermedia* (R Sant.) Vězda, *Mazosia rotula* (Mont) A. Massal and *Strigula concreta* R Sant are new records for India and 21 new distributional records for North-East India. At present, a comparative account of species diversity at state level reveals that Arunachal Pradesh shows maximum species diversity with 65 species, followed by Nagaland with 31 species and Manipur with 8 species Abundance of foliicolous lichens in tropical and subtropical regions implies that these areas are sites of foliicolous lichen diversity

(Key words: foliicolous lichens/ diversity/ North-East India)

Introduction

Lichens strictly growing on living leaves are known as foliicolous lichens. According to Farkus and Sipman[1], "Foliicolous lichens are lichenised fungi colonizing living leaves and leaf like organs (photosynthetic laminae) of plants." Most of them are crustose and found usually on cuticulate leaves both over and under cuticle of upper and lower surfaces (e.g. *Strigula).* Generally more than a single species grows on the same leaf or on different leaves of the same plant. They are prevalent in tropical and subtropical forests in shady to sub-shady places along the banks of streams, lakes, rivulets and riverines on the lower branches of trees, shrubs and under-shrubs. There are about 716 species reported from different regions of world

(Lücking[2]) and they contribute substantially to floristic diversity as well as to the ecosystem management. This group of lichens has its own role in monitoring pollution as they act as bioindicators in tropics (Lücking[3]). They are also fed by small organisms found in their microhabitat.

North-East India, comprising of seven sister states (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram Nagaland and Tripura) and Sikkim, lies between 21°5'-29°30'N latitude and 87°-97°30' E longitude. It covers an area of 2,52,212 sq. km with a forest cover of 1,66,917 sq. km (Anonymous[4]), i.e. about 66% of the total area. The region consists of mountains, plateaus and valleys of various dimensions. Majority of the mountain peaks are in average of 1800 m to 3000 m with Kanchanjanga being the highest (8598 m.) peak amongst all. The variation in the altitude, high rainfall and availability of wind current coupled with moist warm climate are the main factors responsible for rich foliicolous lichen diversity and their luxuriant growth in the region. In spite of its rich foliicolous lichen vegetation the region is not fully explored. Santesson[5] in his monumental work listed only 6 species from the region, i.e. from Assam, Manipur and Meghalaya. After a long gap of 25 years, K Singh[6,7] reported 5 new records of foliicolous lichens from Manipur. Other important contributions from the area are by Sinha and K. Singh[8,9], K. Singh[10], and Sérusiaux and Sloover[11]. More recently, K. Singh and A. Pinokiyo[12] reported 4 species of foliicolous lichens from Arunachal Pradesh as new records to Indian flora. Contributions to the foliicolous lichens from other parts of India were made by A. Singh[13,14,15,16,17,18,19], D. Awasthi and K. Singh[20,21,22], D. Awasthi and Mathur[23], Upreti and A. Singh[24], G. Pant and D. Awasthi[25], Makhija *et al.*[26], Sethy and Patwardhan[27]. D. Awasthi[28] while compiling the micro lichen flora of India, listed about 100 foliicolous lichens including 11 foliicolous lichen species from North Eastern region. K. Singh[29] listed 843 lichens from Eastern Himalaya including 38 foliicolous taxa. Since North-East India is understood as the richest lichenogeographic area of the country (Singh & Sinha[30]) more foliicolous taxa could be expected. Hence, the present study was taken up to provide a detail inventory of foliicolous taxa of North-East India. The paper enumerates all the foliicolous taxa so far known from the region.

Methods

The information presented here is based mainly on recent investigations carried out on the specimens collected by one of the author (K.P.S.) from 1981 to 2000 as well as published records. Identification of specimens has been carried out using well-established lichenological methods (Santesson[5], Grube[31]). Nomenclature of the taxa was updated following Lücking[2]. All the identified specimens are lodged in 'ASSAM' herbarium, Botanical Survey of India, Eastern Circle, Shillong.

Observations

Diversity : The present study indicates that North-East India is the richest area in terms of occurrence of foliicolous lichen species in the country In the present state of knowledge a total of 74 foliicolous lichen species are classified in 29 genera compared to 112 species under 34 genera in the country. It constitutes about 67% of the total foliicolous lichens found in the country and about 10% of world's foliicolous lichen flora. The study also records three foliicolous lichens new to India and 21 species reported for the first time from North- East India However, the present statistics may change considerably as studies on underexplored areas are continued An enumeration of all the taxa recorded so far, is given in Table-I. New records for India are indicated by double asterisk (**) and new distributional records for North-East India by single asterisk (*). A comparative study of state wise distribution of foliicolous taxa in North-East India shows that Arunachal Pradesh exhibits highest species diversity, represented by 65 species, followed by Nagaland with 31 species and Manipur with 8 species. The foliicolous lichens from other remaining North-East states are yet to be recorded. Trichotheliaceae with 15 species is the largest family in the region. Other dominant families include Ectolechiaceae (12 spp.), Strigulaceae (10 spp.), Gomphillaceae (10 spp.), Pilocarpaceae (10 spp.), Opegraphaceae (7 spp.) etc.. Similarly, *Porina* (15 spp.), *Strigula* (9 spp.), *Mazosia* (6 spp.), *Calopadia* (5 spp.), *Byssoloma* (4 spp.), *Fellhanera* (4 spp.) are some of dominant genera found in the region. Interestingly, two species, viz. *Lecidea nagalandica* Sinha and K. Singh and *Phyllobathelium indicum* Sinha and K. Singh are currently known only from Nagaland and Arunachal Pradesh respectively. The occurrence of the high species diversity indicator genus *Badimia* (Lücking[32]) also signifies foliicolous lichen diversity in the region. It is notable that highest species diversity is recorded in tropical moist forests (up to 800 m.) with the dominance of the genera like *Badimia, Gyalectidium, Phyllobathelium, Porina, Sporopodium, Strigula,* etc. forming crowded colonies while, genera like *Bacidia, Calopadia, Fellhanera, Tapellaria, Trichothelium,* etc. are usually found in subtropical forest (up to 2200 m.) forming sparse colonies.

The three new records of foliicolous lichens for India are dealt as follows :

1. Loflammia intermedia (R. Sant.) Vezda, Folia Geobot. Phytotax , Praha, 21: 216. 1986; *Lopadium intermedium* R. Sant., Symb. Bot. Upsal. 12(1): 542, 1952.

The species is characterised by the absence of cephalodia, pale to dark red coloured apothecia with white thick margin, 8-spored asci and submuriform 28-36 x 7-11 µm sized ascospores

Loflammia intermedia (R. Sant.) Vězda, resembles *Loflammia epiphylla* (Fie) Lücking which has singled spored asci and muriform spores. Morphologically it also shows close affinity with *Loflammia grabrielis* (Müll. Arg.) Vezda, but the latter species has transversely septate spores. The species is distributed in the tropical regions of Asia.

Specimens examined : **Arunachal Pradesh** : Upper Subansiri district, Raga Daporizo Road, near Musi-Mugli, Singh & Barua 11931 b, Raga-Daporizo Road, Sadal, Singh & Barua 11938a.

2. Mazosia rotula (Mont.) A. Massal., Neagenea Lichenum: 9. 1854; R. Sant., Symb. Bot. Upsal. 12(1): 113, 1952; *Strigula rotula* Mont., Histoire de l'ilede *Cuba* 9 (2): 142. 1838-1842.

The species is easily characterised by brownish-gray radiating ridges of thallus, immersed apothecia and transversely 3-septate ascospores.

Mazosia rotula (Mont.) Massal. resembles *Mazosia phyllosema* (Nyl.) Zahlbr., but the latter species has smooth thallus. The species is distributed in pantropical regions of the world.

Specimens examined : **Arunachal Pradesh** : Upper Subansiri district, Taliah, in between Sippi and Mengi, near hanging bridge, Singh & Barua 11961b, 11970b, 11976a, 11998c; West Siang district, Keying, Singh 12896p, 12898b, 12900a, .12897 j; Along Kabu forest, Singh 12883.

3. Strigula concreta (Fée) R. Sant., Symb. Bot. Upsal. 12(1): 149.1952; *Craspedon concretum* Fée, *Essai sur les Cryptogames des* éeorees *exotiques officinales :* 94.1824.

The species is characterised by effigurate, irregular, greenish-gray thalline patches having black points, lobes with rounded margins lacking black lines, thread like, cylindrical, 8-spored asci and transversely 2-celled, 9-12 x 2-4 µm sized ascospores.

The species *Strigula concreta* (Fée) R. Sant can easily be differentiated from its closely related species *Strigula nitidula* Mont. by its lobed thalline margin. The species is distributed in the pantropical regions of the world.

Specimens examined : **Arunachal Pradesh** : Debang valley district, way to Mehao Wild Life Sanctuary, Abango to Anda camp, Singh & Barua 11760a, 11762, 11763, 11769a; West Siang district, along Kabu forest, Singh 12885f; along Taniola Basti, Singh 12889; Subansiri district, Leko Basti, Singh 12928a.

Table 1–An enumeration offoliicolous lichens of North-East India

Name of the Genus & (Family)	Name of the Species	Distribution
(1)	(2)	(3)
Asterothyrium Müll. Arg (Asterothyriaceae)	*A pittieri* Müll. Arg.	Ma.
	A rotuliforme (Mill. Arg) Sérus	Ma.
Auluxina Fée (Gomphillaceae)	**A. quadrangula* (Stirton) R. Sant.	AP
	A. uniseptata R. Sant.	Ass.
Bacidia (Müll. Arg.) Zahlbr. (Bacidiaceae)	**B. olivaceorufa* Vainio	AP
Bacidina Vézda (Lecanoraceae)	*B. apiahica* (Müll. Arg.) Vézda	AP.
Badimia Vézda (Pilocarpaceae)	*B. galbinea* (Krempelh.) Vézda	AP
Bapalmuia Sérus. (Pilocarpaceae)	*B. palmularis* (Müll. Arg) Sérus	AP.
Bullatina Vézda & Poelt (Gomphillaceae)	*B. aspidota* (Vainio) Vézda & Poelt	Ass.
Byssolecania Vainio (Pilocarpaceae)	*B. fumosonigricans* (Müll.Arg.) R. Sant.	AP., Na.
Byssoloma Trevisan (Pilocarpaceae)	*B. chlorinum* (Vainio) Zahlbr.	AP., Na.
	B. leucoblepharum (Nyl.)Vainio	AP., Me., Na.
	B. subdiscordans (Nyl.) P. James	AP , Ma., Na.
	B. tricholomum (Mont.) Zahlbr.	AP.
Calenia Müll. Arg. (Gomphillaceae)	*C. conspersa* (Stirton) R. Sant.	Ma., Na.
Calopadia Vézda (Ectolechiaceae)	*C fusca* (Mül. Arg.) Vézda	Na.
	C. nymanii (R. Sant.) Vézda	AP., Na.
	C. perpallidum (Nyl) Vézda	AP.
	C. puiggarii (Müll. Arg.) Vézda	AP., Na.
	C. subcoerulescens (Zahlbr.) Vézda	AP., Na.

Table 1 Contd..

Table 1 Contd.

Chroodiscus (Müll.Arg) Müll Arg (Thelotremataceae)	**C mirificus* (Krempelh) R Sant	AP.
Coenogonium Ehrenb (Gyalectaceae)	**C luteum* (Dicks) Kalb & Lüking	3AP.
Cryptothecia Stirton (Arthoniaceae)	*C. candida* (Krempelh) R Sant	AP
Echinoplacca Fée (Gomphillaceae)	*E epiphylla* Fée	AP., Ma
	E. pellicula (Müll Arg.) R. Sant	AP , Ma.
Fellhanera Vézda (Pilocarpaceae)	*F. bouteillei* (Desm) Vézda	AP., Ma., Na.
	F fuscatula (Mull. Arg.) Vézda	AP., Na.
	F rhapidophylli (Rehm) Vézda	AP., Na.
	F. semecarpi (Vainio) Vézda	AP., Na.
Gyalectidium Müll. Arg. (Gomphillaceae)	*G filicinum* Müll. Arg	AP., Na.
Lecidea Ach. (Lecideaceae)	*L nagalandica* Sinha & K. Singh	Na.
Loflammia Vézda (Ectolechiaceae)	***L intermedia* (R. Sant.) Vézda	AP.
Mazosia Massal. (Opegraphaceae)	**M bambusae* (Vainio) R. Sant.	AP.
	M melanopthalma (Müll Arg.) R. Sant	AP., Na.
	M. phyllosema (Nyl.) Zahlbr.	
	M paupercula (Müll. Arg) R. Sant.	
	***M. rotula* (Mont.) A. Massal.	AP.
	M tumidula (Stirton) Müll. Arg.	AP.
Opegrapha Ach. (Opegraphaceae)	*O. filicina* Mont.	AP., Na.
Phyllobathelium (Müll. Arg.) Müll Arg (Strigulaceae)	*P indicum* Sinha & K. Singh	AP.
Phylloblastia Vainio (Strigulaceae)	*P. dolichospora* Vainio	AP.
Porina Müll. Arg. (Trichotheliaceae)	**P. chrysophora* (Stirton) R. Sant.	AP.
	**P. conica* R. Sant.	AP.
	P. cupreola (Müll. Arg.) Schilling	AP., Na

Table 1 Contd..

Table 1 Contd..

	P epiphylla (Fée) Fée	AP., Na.
	**P imitatrix* Müll Arg	AP.
	**P karnatakensis Makh*	AP.
	**P. limbulata* (Krempelh.) Vainio	AP.
	**P. lucida* R Sant.	AP.
	P monocarpa (Krempelh) Schilling	AP., Na.
	P nitidula Müll Arg.	AP., Na.
	**P pallescens* R. Sant.	AP.
	**P rufula* (Krempelh) Vainio	AP.
	P. trichothelioides R. Sant	AP.
	**P virescens* (Krempelh) Müll. Arg.	AP., Na.
Sporopodium Mont. (Ectolechiaceae)	**S argillaceum* Zahlbr br.	Ap.
	**S phyllocharis* (Mont.) Massal.	AP.
	S. xantholeucum (Müll. Arg) Zahilbr.	AP. Na.
Strigula Fr. (Strigulaceae)	***S. concreta* (Fée) R. Sant	AP.
	**S janeirensis* (Müll Arg.) Lüking	AP.
	S nemathora var. *hypothelia* (Nyl.) R. Sant	Ap.
	**S. nitidula* Mont.	AP.
	**S. orbicularis* Fr	AP., Ass. Na.
	S. smaragdula Fr.	AP. Na.
	S. subelegans Vainio	AP.
	**S. subtilissima* (Fée) Müll Arg.	AP.
	S. phyllogena (Müll Arg.) R C. Harris	Ap., Na.

Table 1 Contd..

Table 1 Contd ..

Tapellaria Müll Arg (Ectolechiaceae)	*T bilimbioides* R Sant	Ap., Na
	T epiphylla (Müll. Arg) R Sant	Ap.
	T molleri (Henriques) R. Sant	Ap
Tricharia Fée (Gomphillaceae)	*T albostrigosa* R Sant	AP , Na.
Trichothelium Mull Arg (Trichotheliaceae)	*T triseptata* R Sant	Ma
	T vainioi R. Sant	Ap , Na
	T epiphyllum Müll Arg.	AP.

**new record for India, * new distributional record from North-East

Abbreviations : Ass.=Assam, AP =Arunachal Pradesh, Ma =Manipur, Me.=Meghalaya, Na = Nagaland

Discussion

Threats and Conservation : The anthropogenic pressure has been the most unwanted factor that has direct impact on natural vegetation. Among the factors, the shifting cultivation commonly practiced by tribal communities in tropical and subtropical forests of the region is the sole factor responsible for depletion of foliicolous lichen rich sites. This causes great threat to many taxa due to burning of virgin forests. Moreover, flood and high water current also destroy natural habitat of these lichens especially along the riverbanks and streams. Thus, suitable measures are required to be formulated to conserve these lichens. At present, it is premature to discuss the conservation aspect of foliicolous lichens in India, because, the information about distribution of species and specific ecological conditions in which they thrive are not available adequately. However, it is observed during survey work that many pockets in lowland forests have rich foliicolous lichen diversity. If these forests in tropical and subtropical areas are conserved as *in situ,* the protection of foliicolous lichens to a great extent will automatically be ensured. Hence, a detailed knowledge of foliicolous lichens and their ecological requirements are essential for planning future conservation strategies.

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Colchicine induced autotetraploid of pigeon pea (*Cajanus cajan*)

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Abstract

Colchicine induced autotetraploid in pigeon pea *(Cajanus cajan)* was produced Tetraploid was compared with the diploid at cytological and morphological level. Induced tetraploid showed vigour for certain morphological characters such as size of leaf, flower, stomata and pollen grains as compared to the diploid. The seed setting was much less in the autotetraploid than in the diploid. Based on the present investigation it is suggested that the size of stomata and pollen grains could be considered as reliable criteria for judging polyploidy in *Cajanus cajan* A considerable reduction in multivalent frequency from Co to C_1 generation was noticed.

(**Keywords**: colchicine induced autotetraploid/*Cajanus cajan*/morphology/cytology)

Introduction

Induced polyploid offers scope to increases the chromatin content of a cell which in turn auto tetraploids are of more economic importance, providing gigas vegetative parts. However, the autotetraploids can be used for developing aneuploids stocks also. Among the few available examples of economically viable varieties resulting from induced autopolyploidy, forage plants autotetraploids of *Cajanus cajan.*

Materials and Methods

One locally adapted variety of pigeon pea *(Cajanus cajan)* was used for induction of polyploidy in present study. To induce polyploidy, apical buds of 100 healthy seedlings of pigeon pea were treated with four different concentrations of (0.025, 0.05, 0.1 & 0.2%) aqueous colchicine solution for 2, 4, 6 and 8 hours using absorbent cotton plug. Treated seedlings were finally transferred to the field. Five mature autotetraploids were studied and compared with their diploids, grown in similar environmental conditions.

Results

Morphology . Chromosome doubling was successfully induced following treatments of apical buds using absorbent cotton plug soaked in 0.2% aqueous colchicine solution for 8 hours. With other concentrations and duration, mentioned above, autotetraploid plants could not be obtained. These plants were similar to diploid plants morphologically and cytologically. Comparative morphological characters of diploids and autotetraploids are summarized in Table-1. The colchicine treated seedlings of pigeon pea had stunted growth. The Co plants differed strikingly in several characters from the diploids (Table 1). Tetraploid plants showed reduction in primary and secondary branches, plant height, length of pod (4.7 cm in 2x, 3.6 cm in 4x), pod set percentage (30 % in 2x and 4 % in 4x) and ovule fertility (87 % in 2x and 25 % in 4x) as compared to the diploids (Table 1). Induced autotetraploid showed increase in size of leaves (Fig. 1), stomata (Figs.2, 3), flower and pod thickness. Average number of seeds per pod was 1.0 in the autotetraploid, where as it was 2.3 in case of diploids. Delayed flowering, as compared to diploids is the characteristic feature of autotetraploid in C_0 as well as in C_1 generation.

Table 1–Comparative morphological observations in diploid and induced tetraploid

Characters	C *cajan* 2x	C *cajan* 4x Co	C. *cajan 4x* C 1
No. of primary branches	6	2	4
No. of secondary branches	14	3	6
Central leaflet -			
Length x breadth (cm)	4 0 x 1.5	5.3 x 2.0	5.5 x 2 0
Length of petiole (cm)	2 1	2.5	2 6
Height of plant (cm)	118	56	75
Days from sowing to bud initiation	92	115	105
Days from sowing to flowering	105	138	131
Days between bud to flower	12	16	14

Table 1 Contd..

Table 1 Contd..

Days between pod initiation to maturity	36	40	38
Size of the standard petal L x B (cm)	1 5 x 1.4	1.8 x 1.6	1.8 x 1.7
Length of style (cm)	1.5	1 8	1.8
Pod length x breadth (cm)	4.7 x 0 8	3 6 x 0 8	3.9 x 0.8
Thickness of pod (cm)	0.7	0.8	0.78
No of chambers per pod	3.1	2	2.4
No of seeds per pod	2 3	1	1.1
Thickness of seeds (cm)	0 41	0.5	0.52
Days to maturity	175	211	207
Pod set %	30	4	9
Ovule fertility %	87	25	50
Stomata frequency	6	4.5	5
Stomata length x bredth (u)	15 x 12	21 x 18	16.8 x 17.5
Pollen fertility (%)	98	82.7	86.31
Fertile pollen size (u)	38.4	44.7	44.4

Cytology : Eleven bivalents (2n = 22) were regularly formed at metaphase-I in diploid *Cajanus cajan.* The Co plants revealed 44 chromosomes. The earliest meiotic stage that could be critically studied was diakinesis. At this stage, one bivalent was seen to be associated with the nucleolus in the diploid plants where as in Co plants two bivalents or one quadrivalent were associated with the nucleolus. Various chromosomal associations (Table 2) as pentavalent, quadrivalents, trivalents, bivalents and univalents were recorded in Co plants (Figs. 4,5). Most of the quadrivalents were ring shaped. Frequency of various chromosome configurations in Co and C_1 plants, is listed in Table-2. Maximum number of eight qudrivalents were recorded in 34.72%

cells. At anaphase-I, 94.08% cells revealed normal separation of chromosomes. Laggards and unequal distribution (Fig. 6) were recorded in 3.84 and 1.92% cells respectively. Regular tetrad formation was observed in 95.41% cells leaving 4.7% cells, wherein micronuclei (Fig. 7) were recorded.

Table 2–Chromosome associations at Metaphase-I in induced autotetraploid of pigeon pea

Generation	Total PMC studied	Chromosome association at M-I	% of PMCs
Co	46	8 IV + 6 II	34.72
		5 IV + 12 II	26.04
		4 IV + 1 III + 11 II + 3 I	8.69
		1 V + 4 IV + 3 III + 7 II	4 34
		3 IV + 16 II	17.39
		2 IV + 17 II + 2 I	8.69
Cl	34	8 IV + 6 II	11 76
		5 IV + 12 II	17 64
		4 IV+ 1 III + 11 II + 31	5.88
		4 IV+ 14 II	14.7
		3 IV + 16 II	14.7
		2 IV + 18 II	11.76
		2 IV + 16 II +4 I	8.82
		2 IV + 17 II + 2 I	5.88
		22 II	8 82

Pollen fertility in Co plants was 82.7, while diploids showed 99.2% (Table 1). An increase in fertile pollen size of tetraploids over those of dioploids (Fig. 8,9) was recorded. Meiosis in C_1 plants (Figs. 10,11) revealed reduction in the number of quadrivalents per cell and increase in pollen fertility as compared to Co plants. Mitotic studies from the seeds of C_1 plants revealed 44 chromosomes (Fig. 12).

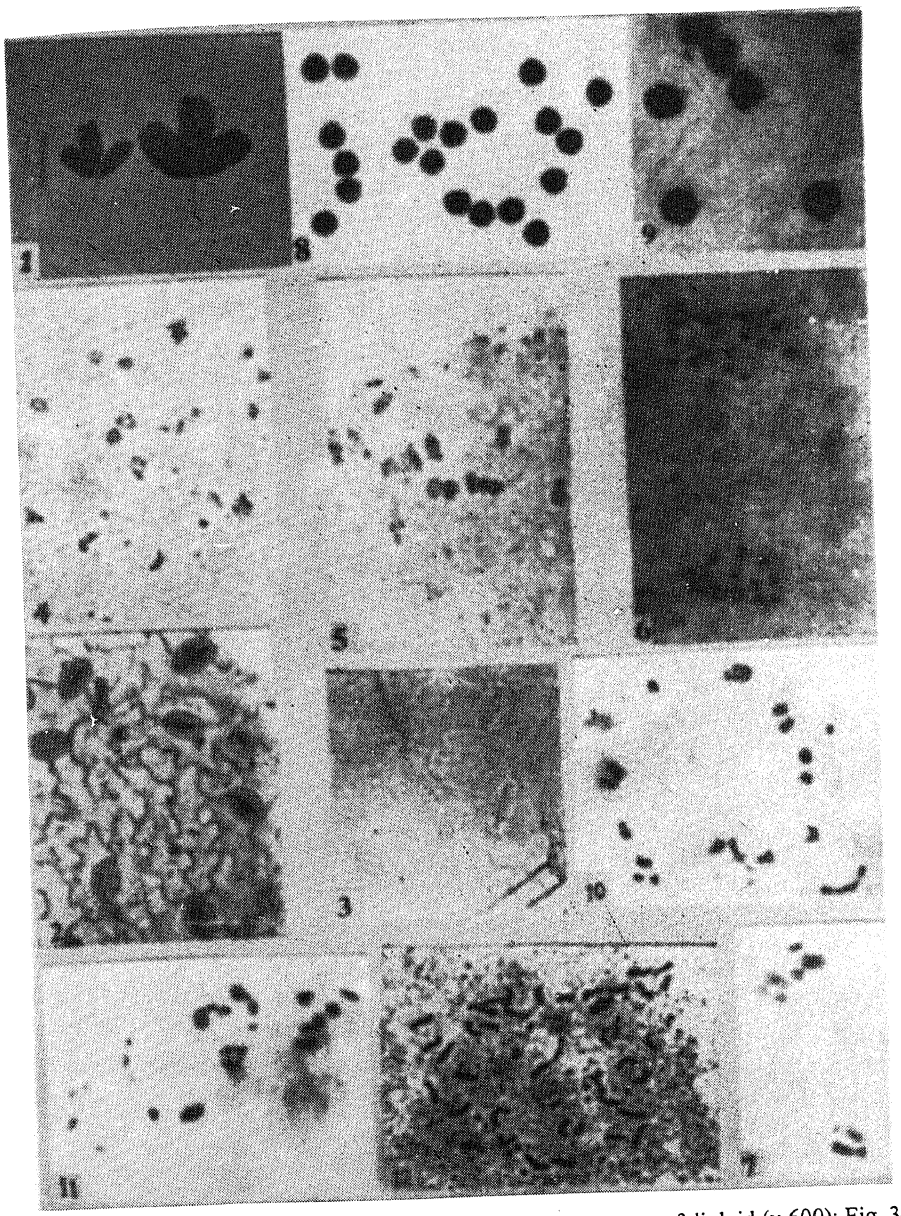

Fig. l– Leaf of diploid and tetraploid *Cajanus cajan;* Fig. 2– Stomata of diploid (x 600); Fig. 3– Stomata of tetraploid (x 600); Fig 4–3 IV s + 16 IIs at Metaphase-l of Co plant (x 1500); Fig. 5 –5 IVs + 12 IIs at M-l of C_0 plant (x 1500); Fig. 6– Laggards and unequal distribution at Anaphase-l of Co plant (x 1500); Fig. 7– Micronuclei with normal tetrads (x 600); Fig. 8– Pollen grains of diploid plant (x 600); Fig. 9– Pollen grains of Co tetraploid (x 600); Fig. 10– 4 IVs + 1 III + 11 IIs + 3 Is at late diakinesis of C_1 plant showing one quadrivalent attached to the nucleoli (xI500); Fig. 11– 2 IVs + 17 IIs + 2 Is at M-I of C_1 plant (x 1500); Fig. 12– 44 somatic chromosomes of C_1 plant at metaphase (x 1500).

Discussion

Following the discovery of the use of colchicine for the production of polyploids, there was considerable enthusiasm among the plant breeders to induce polyploidy directly in the improvements of plants and to utilize gigas characters. There is a small group of material in which the reaction of chromosome doubling is favourable. Such material have particularly low chromosome number indicating that the plant concerned is not already either primary or secondary polylpoids. In the present experiments, for induction of polyploidy, 4-6 days old seedlings were treated with colchicine solutions through absorbent cotton plug. Seedlings has the advantage that root system need not be effected and the shoots alone can be treated. Seedlings could not survive at increased concentrations and duration. At lower concentration and duration, the survived seedlings were found to be diploid after cytological examinations. Success in polyploidy with 0.2% colchicine is also reported previously in *Cajanus cajan*[6].

The induced autotetraploid of pigeon pea *(Cajanus cajan)* exhibited typical effect of autotetraploidy with regard to morphological characters. Exhibition of highly stunted growth in initial stages was the uniform feature of seedlings survived after colchicine treatment in pigeon pea. One of the effects generally associated with induction of polyploidy was gigas nature of vegetative as well as floral parts in the polyploids. The induced autotetraploids showed delayed flowering and maturity, which may be attributed to slower rate of metabolic activities and cell division in the autotetraploids. The increase in cell size is well known effect of polyploidy and increase in the size of stomata and pollen is normally associated with polyploidy. Such increase was pleiotropic and resulted in an increase in the determinate organ like floral parts and seeds. In the present study, a wide variation in pollen size was recorded.

Induced autotetraploids exhibited fairly good pollen fertility. Yet, in autotetraploids the pod setting (4.0%) and seed setting (1.0 seeds / pod) were much lower than the diploids, which may be due to high flower shedding in these autotetraploids. In the present study, most of the Co plants showed rudimentary pods with abortive seeds, which could not germinate. The development of abortive seeds indicated that some physiological changes in the ovarian tissue might be responsible for this or it could be due to pre/post fertilization disturbances leading to abnormal embryo development.

With regards to the previous findings[7] that in all the autotetraploid plants about 66% of the chromosomes are, on an average, associated as quadrivalents. this was however not observed in autotetraploids of *Cajanus cajan* developed in this study. Due to the presence of four homologous chromosomes, multivalent formation is expected in all the PMCs of all autotetraploids. but the formation of bivalents in many PMCs suggested that presence of more than two homologous chromosomes is not the only requirements for multivalent formation. Most of the cells had lower quadrivalent frequency. Occasionally association of more than four chromosomes such as pentavalent in 4.38% cells, was noticed for which no clear explanation can yet be given. Low quadrivalent frequency has been reported in *Cajanus cajan* by earlier workers also[8].

In C_1 generation, considerable improvement in pod and seed setting over those of Co plants could be attributed to decrease in the frequency of multivalent from C_0 to C_1.This downward trend in quadrivalent frequency may be due to the stabilization of autotetraploidy..

Univalents at metaphase 1, may be considered as the most important type of irregularity because of the tendency of these unpaired chromosomes to lag and divide equationally at anaphae -1 and to be left in the cytoplasm at telophase 1 and 2. These univalents were led to the formation of aneuploid gamets and micronuclei. In the course of present study, the cytological studies in the PMCs did not reveal much meiotic irregularities at anaphase except lagging chromosomes and spindle abnormalities which were also of rare occurrence.

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Complexes of transition metals with tridentate N-(2-thiazole) benzamide-2'-carboxylic acid and oxine and their antimicrobial studies

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Abstract

Complexes of the type $[ML_2]$ and $[MLL'.H_2O]$ [where M = Mn(II), Co(II), Ni(II), Cu(II) and Zn (II); HL = N-(thiazole)-2'-carboxylic acid; and L' = oxine] have been synthesized The complexes are octahedral in nature. The synthesized ligand behaves as tridentate OON donor. The characterization of the complexes has been done on the basis of analytical, molar conductance, magnetic susceptibility, molecular weight, infrared and electronic spectral data. Antibacterial activity of these ligands and their metal complexes has been determined on gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria at 37 °C and antifungal activity has been determined on common fungi viz. *Aspergillus niger, Aspergillus nidulans* and *Candida albicans* at 28 °C. It has been found that the biocidal activity of these ligands increases on being coordinated with suitable metal ion

(**Keywords**: I.R./electronic spectra/mixed ligand complexes/ternary complexes/metal chelates)

Introduction

Oxine, heterocyclic acids and their derivatives have long been established as potential antimicrobial agents and drugs[1,2]. The information about the role of metal complexes in biological systems is of immense importance. Reports exist in the literature about the N, S, and O donor ligands and their transition metal complexes which play an important role in the biocidal action. Their metal complexes have been found more biologically active in comparison to either the free ligands or the involved

metal ions[3,4]. Similar observations have been noted on the oxine[5] and its transition metal complexes[6].

In view of the biological importance of the thiazole moiety, several workers have studied the coordination behavior of simple and substituted thiazoles with transition[7-10] and non transition metal ions[11]. In continuation of our earlier work[12], it was therefore, thought worthwhile to undertake studies on binary and ternary metal complexes involving N-(2-thiazole) bezamide-2'-carboxylic acid and oxine as ligand.

Materials and Methods

All the used chemicals were of analytical reagent grade.

Synthesis of N-(2-thiazole) benzamide-2 '-carboxylic acid and its metal chelates :

N-(2-thiazole) benzamide-2'-carboxylic acid (TBCA) has been synthesized by refluxing (5 hours) the equimolar amount or phthalic anhydride and 2-amino thiazole. The compound thus obtained was filtered, washed with benzene and recrystallised with acetone (m.p. 184 °C). The metal complexes have been synthesized as by the procedure reported elsewhere[12]. Their purity has been checked by TLC.

Physical measurements :

The infrared spectra of the ligands and their metal complexes were recorded on Perkin-Elmer-521 spectrophotometer (range 4000-200 cm^{-1}). The electronic spectra of metal complexes were recorded on Cary-14 spectrophotometer (range 200 - 760 nm) using DMSO as solvent. Molar conductance of the complexes was measured (in DMSO solution) using Toshniwal Conductivity Bridge. Elemental analyses were carried out by microanalytical technique and metal contents were estimated by standard methods[13]. The molecular weight of the compounds was determined by cryoscopic method in DMSO. Magnetic measurements were carried out at room temperature by Gouy's method. The values were corrected for diamagnetism by applying Pascal's constant. TGA was carried out at RSIC, Nagpur in nitrogen atmosphere at the heating rate 15 °C min^{-1}.

Results and Discussion

Elemental analyses, molecular weight determination and conductance measurement :

The complexes are thermally stable and insoluble in water. The molar conductance of the complexes in DMSO (10^{-3} M) solution was found in the range

0.02-2.0 ohm^{-1} cm^2 mol^{-1}, indicates the non-electrolytic nature[14]. The 1 : 2 type of stoichiometry in the case of binary complexes and 1: 1 : 1 type of stoichiometry in the case of ternary complexes were concluded from their elemental analyses and molecular weight measurement data (Table 1). Thermogravimetric analysis and infrared spectra of the complexes confirm the presence of water molecule.

Thermogravimetric analysis :

On gradual heating from room temperature, the complexes get dehydrated completely in the temperature range 110-165 °C. The dehydrated complexes decompose in the temperature range 280-470 °C. The resultant products were the metal oxides in all cases.

Infrared studies:

The appearance of new bands around 3,580-3,450 cm^{-1} and 1,575 cm^{-1} indicate the antisymmetric and symmetric OH stretching and H-OH bending modes. The amide I band appearing at 1,735 cm^{-1} in case of ligand (TBCA) has shifted to lower wave number (25 cm^{-1}) in the complexes, indicating coordination through carbonyl oxygen of the amide group[15]. A slight decrease in the NH (amide II) band is observed, supporting the involvement of carbonyl oxygen in bonding. The NH band at 3,150 cm^{-1} remain unaffected in complexes (involving TBCA as ligand), indicating that imine nitrogen is not forming any bond with metal ion. The carboxylic group stretching frequency of TBCA is lowered from 1,680 to 1,660 cm^{-1} showing the coordination of carboxylic group[16]. A band in the region 1,535-1,475 cm^{-1} due to thiazole ring in TBCA indicates non-involvement of thiazole sulfur in bonding[17]. The OH (phenolic) stretching frequency at 3,270 cm^{-1} in free oxine is absent in the spectra of M[TBCA][OX].H_2O complexes. This indicates the involvement of phenolic group in complexation. The strong bands in the region 1,175-1,125 cm^{-1} depict the presence of coordinated oxine[18]. The appearance of new bands in the region 480-460 and 430-400 cm^{-1}, is probably due to the formation of M-O and M-N bonds respectivel[19]. No M-S bond could be observed in complexes exhibiting that S is not taking part in complexation[20].

Electronic spectra and magnetic studies :

The magnetic moments of Mn(II), Co(II), Ni(II) and Cu(II); complexes calculated from corrected magnetic susceptibility and electronic spectral data are given in Table 1 and 2. The observed magnetic moment of the complexes favours the octahedral

geometry around the metal ion[21]. Three bands around 19,500, 23,400 and 28,800 cm^{-1} are due to the transitions $^6A_{1g} \rightarrow {}^4T_{1g}$ (G), $^6A_{1g} \rightarrow {}^4E_g$, $^4A_{1g}$ (G) and $^4A_{1g} \rightarrow {}^4E_g$ (D) for manganese complexes supporting the octahedral geometry[22]. Three bands observed in the spectra of cobalt complexes around 8,300, 16,300 and 22,000 cm^{-1}, may be due to $^4T_{1g} \rightarrow {}^4T_{2g}$(F), $^4T_{1g} \rightarrow {}^4A_{2g}$(F) and $^4T_{1g} \rightarrow {}^4T_{1g}$(P) transitions, respectively, supporting the octahedral environment. Three bands have been observed at ~8,500, 14,200 and 26,200 cm^{-1} in the spectra of nickel complexes assignable to three spin allowed transitions from $^3A_{2g} \rightarrow {}^3T_{2g}$ (F), $^3A_{2g} \rightarrow {}^3T_{1g}$ (F) and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) respectively. This supports octahedral geometry[22]. The electronic spectra of the copper complexes show only band around 14,000 cm^{-1} due to $^2E_g \rightarrow {}^2T_{2g}$ transitions[22]. The ligand field parameters Dq, B, β, LFSE and % covalency have been evaluated (Table 2) for the studied complexes. The Racah interelectronic repulsion parameter is less than free ion value suggesting considerable covalent character of the bonds. The calculated values of ν_2/ν_1 ratio is within the range expected for octahedral environment[23].

Table 1–Element analyses, magnetic moment and molecular weight data of ligand and metal complexes.

S No	Compound	Analysis (found/calculated)					μeff B.M. 303°K	Molecular weight (found/calcd.)
		C	H	N	S	M		
1.	TBCA	53.25	3.27	11.24	12.87	...	...	241
		(53 22)	(3.25)	(11.28)	(12.92)	...		(248)
2	$Mn[TBCA]_2$	48.12	2.61	10 23	11.72	9.89	4.91	538
		(48.09)	(2.57)	(10.15)	(11.67)	(9.99)		(549)
3.	$Co[TBCA]_2$	47 29	2 58	10 15	11.51	10.58	4 95	542
		(47.74)	(2.55)	(10.12)	(11.58)	(10.65)		(553)
4	$Ni[TBCA]_2$	47 72	2.57	10 08	11.61	10.55	3.09	545
		(47.76)	(2.55)	(10.13)	(11.59)	(10.61)		(553)
5.	$Cu[TBCA]_2$	47 29	2.51	10 08	11.51	11.31	1 96	549
		(47.35)	(2.53)	(10.04)	(11.49)	(11.38)		(558)
6.	$Zn[TBCA]_2$	47.21	2.55	10.53	17.09	11.62	Diam	551
		(47.19)	(2.52)	(10.01)	(17.14)	(11.68)		(559)

Table 1 Contd...

Table 1 Contd...

7	Mn[TBCA] [OX] H_2O	51 82	3.19	9 09	6 83	11 77	4.97	456
		(51 73)	(3.25)	(9 05)	(6 90)	(11 83)		(464)
8	Co[TBCA] [OX] H_2O	51 54	3.19	8 91	6.71	12 63	5.05	455
		(51.29)	(3 23)	(8 97)	(6 68)	(12 58)		(468)
9.	Ni[TBCA] [OX].H_2O	51 35	3.23	8.92	6.79	12.43	3 13	460
		(51 31)	(3.22)	(8.97)	(6 85)	(1254)		(468)
10	Cu[TBCA] [OX].H_2O	50 11	3 22	8 85	6 82	13.37	2 02	466
		(50.09)	(3.19)	(8.88)	(6 78)	(13.43)		(473)
11.	Zn[TBCA] [OX] H_2O	50 58	3 21	8 90	6.69	13 71	Diam	465
		(50 59)	(3.18)	(8.85)	(6 75)	(13.77)		(474)

Table 2–Electronic spectral data of metal complexes

S. No.	Complex	Band Maxima	Dq	B	β	LFSE	ν_2/ν_1	% Covalency
		cm^{-1}	cm^{-1}	cm^{-1}		$kcal.mol^{-1}$		(δ)
1.	$Mn[TBCA]_2$	19,245	702	710	0.739	.	..	35 320
		23,876						
		28,850						
2.	$Co[TBCA]_2$	8,530	783	821	0.845	13.422	1.918	18.343
		16,366						
		21,551						
3.	$Ni[TBCA]_2$	8,586	858	972	0.933	29.417	1 644	7.181
		14,120						
		26,230						

Table 2 Contd. .

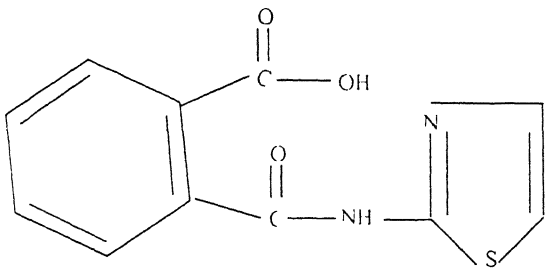
Table 2 Contd .

4	$Cu[TBCA]_2$	14,044	1,404	.	.	24.068		...
5.	$Mn[TBCA]\ [OX]\ H_2O$	19,722	732	806	0 839			19 189
		23,238						
		28,880						
6	$Co[TBCA]\ [OX].H_2O$	8,273	803	909	0 936	.	...	6 837
		16,310						
		22,145						
7	$Ni[TBCA]\ [OX]\ H_2O$	8,482	848	996	0 956	29.074	1 678	4.602
		14,238						
		26,155						
8	$Cu[TBCA]\ [OX]\ H_2O$	14,140	1,414	...	...	24 240	..	.

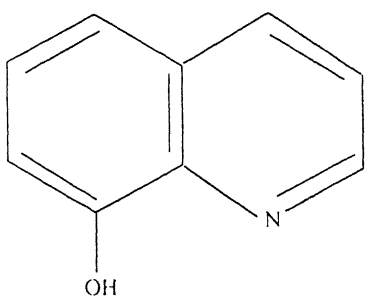
On the basis of elemental analyses, infrared spectra, molar conductance and molecular weight data, the zinc complexes were proposed to have an octahedral geometry. The probable structure has been given in Fig 1.

Antibacterial and antifungal activity :

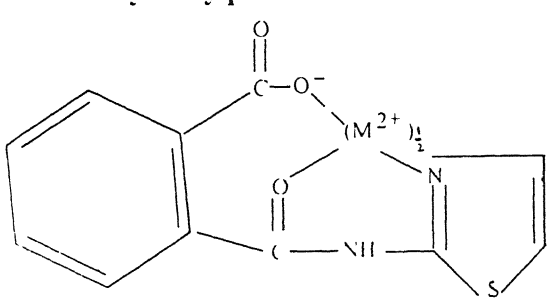
Serial dilution method[24,25] was adopted to study the antibacterial and antifungal activity of the compounds. A close and comparative study of Table 3 reveals that the ligands are active against the bacteria and fungi used. In these investigations, it is found that the activity of oxine is enhanced when it is chelated with suitable metal ion. It has been observed that 1:2 chelate of metal-oxine penetrates the cell and dissociates 1:1 half chelate and free oxine. The half chelate would become the toxic entity. Gershon and Permegiani[4] supported the above mechanism and indicated that biocidal activity of metal oxine is not due to the release of oxine within the cell but due to the dissociated 1:1 complex such as that reported[1]. The comparable activity of the ternary complexes to that of binary complexes may be due to the dissociation of the complexes at the site of action.



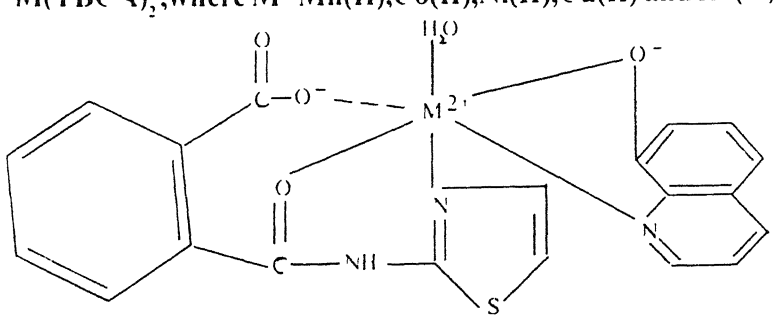
N-(2-thiazole)-2'-carboxylic acid (TBCA)



8-Hydroxyquinoline



$M(TBCA)_2$, where M=Mn(II),Co(II),Ni(II),Cu(II) and Zn(II)



$M(TBCA)(OX).H_2O$, where M=Mn(II),Co(II),Ni(II),Cu(II)& Zn(II)

Fig 1

Table 3–Antibacterial and antifungal activity of the ligands and metal complexes* .

S No	Compound	Bacteria (μmol/L)		Fungi (μmol/L)		
		S aureus	*E coli*	*A niger*	*A nidulans*	*C albicans*
1.	OX	290 02	277 48	292.91	315 44	319 97
2.	TBCA	255 86	230 68	238 47	277 49	235 42
3.	$Mn[OX].H_2O$	100 13	99.67	86 13	90 24	91 05
4.	$Co[OX].H_2O$	66.97	52.52	39.53	54 29	56 17
5.	$Ni[OX]\ H_2O$	79.68	83.20	54 49	66.10	73 91
6.	$Cu[OX]\ H_2O$	99 77	96.91	70 54	82 83	89 15
7	$Zn[OX].\ H_2O$	102 52	102.95	91 24	98 09	92.01
8.	$Mn[TBCA]_2$	120.25	111.58	101.68	103 86	105.38
9.	$Co[TBCA]_2$	79 12	76 51	73.28	70.11	72 .46
10	$Ni[TBCA]_2$	100.29	91.05	83 87	80.51	81 66
11.	$Cu[TBCA]_2$	112.59	100.12	95.24	92.12	91 23
12.	$Zn[TBCA]_2$	129 39	116.25	113 24	115.58	114.20
13.	$Mn[TBCA][OX].H_2O$	42 11	38.46	36 56	40 12	40.60
14	$Co[TBCA][OX].H_2O$	28 05	24.18	23.13	25.46	26 69
15.	$Ni[TBCA][OX].H_2O$	31.28	30.43	29.65	29.87	30 38
16.	$Cu[TBCA][OX].H_2O$	38.55	37.13	31.24	31 36	34 54
17.	$Zn[TBCA][OX].H_2O$	45.23	40 56	38 12	42.65	41.87

TBCA = N-(2-thiazole) benzamide-2'-carboxylic acid; OX = Oxine

*Values are minimum inhibitory concentration (MIC) in μmol/L.

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Antibacterial properties of some new copolymer resins synthesized from 1-[2,4-dihydroxyphenyl]-2-phenyl ethanone and 1-[2,4-dihydroxyphenyl] propanone

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Abstract

Antibacterial properties of the homo and copolymer resins, synthesized from 1-[2,4-dihydroxyphenyl]-2-phenyl ethanone (2,4-DHPPE) and 1-[2,4-dihydroxyphenyl]- propanone (2,4-DHPP) with substituted benzoic acids (comonomers) and formaldehyde or furfural (condensing reagents) in the presence of acid or base catalysts, have been evaluated against four enteric bacteria *(Escherichia coli, Serretia* sp , *Bascillus subtilis* and *Staphylococcus aureus)* All the homo resins and some formaldehyde based copolymer resins were devoid of antibacterial activity. All furfural based and a few formaldehyde based copolymer resins were effective against both Gram-positive and Gram-negative bacteria, with a degree of variation in their activity. 2,4-DHPPE-formaldehyde-orthohydroxybenzoic acid copolymer resin was selective and active against Gram-negative bacteria only. 2,4-DHPP/2,4-DHPPE-formaldehyde-parachlorobenzoic acid and 2,4-DHPPE-furfural-parachlorobenzoic acid copolymer resins were highly potent antibacterial agents surpassing even the streptomycin sulphate standard employed 2,4-DHPP/2,4-DHPPE-furfural-paraaminobenzoic acid and 2,4-DHPPE-furfural-orthohydroxybenzoic acid copolymer resins were equipotent with that of the antibiotic streptomycin.. In general furfural based copolymer resins were more toxic than formaldehyde based resins Similarly copolymer resins prepared using acid catalyst were more toxic than those prepared from base catalyst. Gram-negative bacteria were comparatively more sensitive towards the resin copolymers than Gram-positive bacteria

(**Keywords** : 1-[2,4-dihydroxyphenyl]-2-phenyl ethanone and 1-[2,4-dihydroxyphenyl] propanone copolymer resins/antibacterial properties/enteric bacteria/parachlorobenzoic acid based copolymer resins/potent antibacterial agents).

Introduction

Many natural and some synthetic polymers are subject to attack by biological agents and at times may be a source of nutrients. Such action depends on the susceptibility of the polymer to penetration and degradation. The degree of susceptibility, the use of the material, costs and other factors govern the application of protective treatment. Biologically active compounds used as preservatives, control degradation in several ways: those that cause the death of the degradative organism are termed *biocidal.* Many chemical agents, however, may inhibit the reproduction and growth of organisms without causing their death. These may be exemplified in the two common classes of control agents known as *bacteriostats* and *fungistats.*

Polymers may display biological activity even they do not contain biologically active moieties or repeating units. Normally, the monomers of such polymers of their dimer models do not show the same activity, which confirms that the biological effect is polymer-specific and owing to the respective structure.

The polymer back-bone influences some of the most important biological properties of polymers with bound moieties. Hence, polymers with antimicrobial activity have received special attention[1-4]. Copolymer resins, prepared by condensing chloro/hydroxy/amino and carboxylic derivatives of aromatic compounds and formaldehyde / furfural are known to be effective against wide spectrum of microorganisms such as bacteria, algae, fungi etc. Ciampa and co-workers[5,6] have investigated the biological activity of many polymeric resins incorporating salicylic acid as one of the component. Nayak *et al.*[7-12] have studied the antibacterial activity of a number of resin copolymers derived from a spectrum of organic compounds such as substituted acetophenones, resacetophenones and benzoic acids etc., using formaldehyde and furfural as condensing agents, and observed that some of the copolymer resins were potent antibacterial agents.

In view of the above facts, it was considered worthwhile to study the antibacterial property of some newly synthesized homo and copolymer resins, which was not reported so far. In the present paper, preparation of some new homo and copolymes resins, using 2,4-DHPPE and 2,4-DHPP (monomers), formaldehyde and furfural (condensing reagents) and substituted benzoic acids (co monomers) in the presence of acid or base catalysts, and their antibacterial activity are discussed.

Materials and Methods

Substituted benzoic acids (E.Merck, India) were used as co-monomers after further purification, formaldehyde (B.D.H.) and furfural (S.D. Fine Chemicals Ltd. Boisar, India) were used as such. 1-[2,4-dihydroxyphenyl]-2-phenyl ethanone (2,4-DHPPE) and 1-[2,4-dihydroxyphenyl]propanone (2,4-DHPP) were prepared from resorcinol, $ZnCl_2$ and propionic acid using standard procedures[13,14] and their purity was checked with mixed melting point, Co- TLC and spectral analysis. Solvents and other chemicals used were of AnaiaR grade.

Synthesis of homo and copolymer resins: A mixture of monomer (0.01 mole, 2,4-DHPP or 2,4-DHPPE), condensing reagent (0.05 mole, formaldehyde or furfural), and comonomer (0.01 mole, ortho or para substituted benzoic acids in case of copolymer resins) was taken in a R.B. flask. Catalyst (2 ml of conc. HCI or 2 ml of 10 N aq. NaOH solun) was added slowly to the reaction mixture. The contents were refluxed to 100-120°C for 6-8 hrs in an oil-bath with periodical shaking. After completion of the reaction, the mixture was poured into ice cold water, the separated solid mass was filtered and washed with hot water to remove unreacted reactants. Finally the polymer was washed with alcohol, dried at vacuum and used for physico-chemical analysis and antibacterial activity.

Antibacterial Assay: The antibacterial activity of a compound is expressed as its ability to inhibit the growth of bacteria in nutrient medium. The paper-disc method of Vincent and Vincent[15] was used in the present investigations.

Antibiotic sensitive of four homoresins and twenty copolymer resins were evaluated against four bacteria viz., *Bacillus subtilis*, *Staphylococcus aureus* (both Gram-positive), *Escherichia coli* and *Serretia* sp. (both Gram-negative) isolated from clinical specimen, following the agar diffusion method of assay[16,17].

The test organisms were maintained on using nutrient agar and stored in a refrigerator. Bacterial inoculum was prepared by transferring a loopful of stock culture to 100 ml nutrient broth (beaf extract, peptone glucose, agar 15 g and 1 litre distilled water) in a 250 ml conical flask. The flasks were incubated at 37 ± 1°C for 24 hours before the experimentation.

Solutions of the test resins were prepared by dissolving 6-9 mg each indimethyl sulphoxide (1 ml DMSO; Analar grade). A reference standards were made by dissolving accurately weighed quantity of sodium salt of benzyl pencillin and streptomycin sulphate in sterile distilled water, separately to serve as standard antibiotics for Gram-positive and Gram-negative bacteria respectively.

The nutrient agar medium was sterilized by autoclaving at 121 °C (15 lb/sq. inch) and poured into sterilized petri-plate (10 cm diameter). The petri plates thus prepared were inoculated with bacteria (6 ml). The plates were left at room temperature to allow the solidification. Whatman filter paper discs of 6 mm diameter were made with a sterile punching machine. Accurately 0.1 ml (6 mg/ml or 9 mg/ml; 600 μg/disc or 900 μg/disc respectively) of the test solution was added, ascetically, to each disc, labelled accordingly and dried at room temperature for 24 hours. In each petri plates, prepared as above, the dried paper discs containing test compound were placed with a sterile forcep and incubated at 37 ± 1 °C for 2 days. At the end of 48 hrs incubation, the degree of sensitivity was determined by measuring the zone of inhibition, which is the area around the disc that did not have bacterial growth and represented in terms of zone of inhibition and diameter (in mm) after conversion.

Test compounds were employed at two concentrations i.e. 600 and 900 μg/disc in DMSO. Simultaneously paper discs impregnated with DMSO solvent and dried at room temperature for 24 hours, were used as controls. Experiments were repeated at least thrice. As the difference among the replicates was statistically insignificant, average of these was taken. The observations made are presented in Table 1.

Table 1–Antibacterial assay of homo and copolymer resins

S. No.	Name of the resin/resin copolymer	Conc. (μg/ml)	Diameter of inhibition zone (mm)			
			E coli	*Serretia* sp.	*B subtilis*	*St aureus*
[1]	[2]	[3]	[4]	[5]	[6]	[7]
1.	2, 4-DHPP- Formaldehyde	600	NA	NA	NA	NA
		900	NA	NA	NA	NA
2	2, 4-DHPP-Formaldehyde-	600	NA	NA	NA	NA
	orthochlorobenzoic acid	900	NA	NA	NA	NA
3	2, 4-DHPP-Formaldehyde-	600	6 15	7.00	8.00	8.00
	parachlorobenzoic acid	900	9.00	10.10	9.00	10.10
4	2, 4-DHPP- Formaldehyde-	600	3.70	3.14	4.50	3.14
	orthohydroxybenzoic acid	900	4.50	3.70	6.50	4.50

Table 1 Contd..

Table 1 Contd.

5	2,4- DHPP- Formaldehyde-	600	NA	NA	NA	NA
	orthoaminobenzoic acid	900	NA	NA	NA	NA
6	2,4- DHPP-Formaldehyde-	600	NA	NA	NA	NA
	paraaminobenzoic acid	900	NA	NA	NA	NA
7	2,4-DHPP-Furfural	600	NA	NA	NA	NA
		900	NA	NA	NA	NA
8	2,4-DHPP-Furfural-	600	2 50	3.14	2.50	5 30
	orthochlorobenzoic acid	900	8 00	6 15	3.70	6 50
9	2,4- DHPP-Furfural-	600	3.60	3 90	3.70	3.14
	parachlorobenzoic acid	900	4.50	4 90	4.50	3.70
10.	2,4-DHPP-Furfural-	600	3 14	5.30	4 50	4 50
	orthohydroxybenzoic acid	900	6.50	6 50	6.51	7 02
11	2,4-DHPP-Furfural-	600	1.13	2 50	3.13	3.70
	orthoaminobenzoic acid	900	5 30	7 00	4 50	6.15
12.	2,4-DHPP-Furfural-	600	4.50	4.50	3.70	3 14
	paraaminobenzoic acid	900	6 50	5.30	4 50	3.70
13.	2, 4-DHPPE-Formaldehyde	600	NA	NA	NA	NA
		900	NA	NA	NA	NA
14.	2,4- DHPPE- Formaldehyde-	600	NA	NA	NA	NA
	orthochlorobenzoic acid	900	NA	NA	NA	NA
15a.	2,4-DHPPE-Formaldehyde-	600	0.78	3.14	4.50	2 01
	parachlorobenzoic acid (base catalysed)	900	1.50	3.70	5.30	3 70
15b.	2,4-DHPPE-Formaldehyde-	600	8.30	4.50	8.14	4 50
	parachlorobenzoic acid	900	10.10	9.00	8.00	11.30
	(acid catalysed)					

Table 1 Contd..

Table 1 Contd

16	2,4- DHPPE- Formaldehyde-	600	0 50	0 20	NA	NA
	orthohydroxybenzoic acid	900	0 20	1 13	NA	NA
17.	2,4- DHPPE-Formaldehyde-	600	NA	NA	NA	NA
	orthoaminobenzoic acid	900	NA	NA	NA	NA
18	2,4- DHPPE- Formaldehyde-	600	0 50	0 20	2 50	3.17
	paraaminobenzoic acid	900	0 20	2 50	2 80	3.70
19	2,4-DHPPE-Furfural	600	NA	NA	NA	NA
		900	NA	NA	NA	NA
20	2,4-DHPPE-Furfural-	600	6.51	3.70	4 50	2.50
	orthochlorobenzoic acid	900	7.00	4.50	5.30	6 50
21.	2,4-DHPPE-Furfural-	600	9 00	5.30	4.50	5.30
	parachlorobenzoic acid	900	10.10	6 50	7 00	8 50
22	2,4-DHPPE-Furfural-	600	6.50	5.30	3.14	4.50
	orthohydroxybenzoic acid	900	7.00	7.00	3.70	8.00
23.	2,4-DHPPE-Furfural-	600	3.70	3.70	7.00	6.50
	orthoaminobenzoic acid	900	5.30	6.50	7 50	7.00
24.	2,4-DHPPE-Furfural-	600	5.03	3 10	7.00	6.50
	paraaminobenzoic acid	900	6.50	5.01	7.50	7.00
25.	Streptomycin (Reference)	600	4.32	4.18	3 28	3.32
26.	Benzyl pencillin (Reference)	600	--	--	3 28	3.32

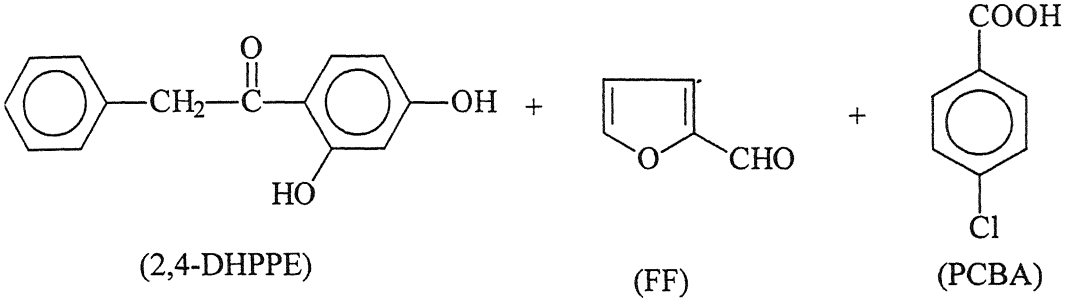
NA = No Activity

2,4-DHPP = 1-[2,4-Dihydroxyphenyl] propanone

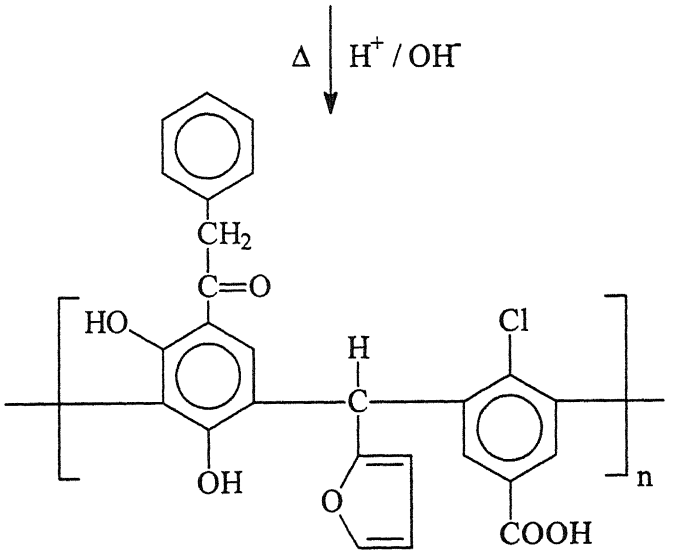
2,4-DHPPE = 1-[2,4-Dihydroxyphenyl]-2-phenyl ethanone

Results and Discussion

The polycondensation reaction of 1-[2,4-dihydroxyphenyl]-2-phenyl ethanone (2,4-DHPPE) with furfural (FF) and parachlorobenzoic acid (PCBA) may be represented as follows :



(2,4-DHPPE) (FF) (PCBA)



(2,4-DHPPE-FF-PCBAresin copolymer)

A large number of homo and copolymer resins were prepared using different combinations of monomers (2,4-DHPPE/2,4-DHPP), condensing reagents (formaldehyde/furfural) and comonomers (ortho and para substituted benzoic acids, in case of copolymer resins), in the presence of acid or base catalysts.

All the prepared homo and copolymer resins were characterised by spectral analysis (FTIR, ^{1}H NMR), solubility characteristics, molecular weight determination and thermal analysis. The solubility behaviour of the resins were determined by using solvents of varying solubility parameter. All the resins were freely soluble in DMSO, $CHCl_3$ and THF; sparingly soluble in acetone and 2-butanol; insoluble in ether, benzene, toluene and water. Number-average molecular weight ($\overline{Mn}$) of these resins was determined conductometrically following the method of Chatterjee *et al.*[18-20] and the calculated $\overline{Mn}$ n for all the resins was below 3000.

Perusal of Table 1, reveals that all homo resins (Sl. No. 1, 7, 13 and 19) and a few copolymer resins (Sl. No. 2, 5, 6, 14 and 17) were lacking antibacterial activity as they failed to inhibit the growth of the bacteria screened. But all furfural based copolymer resins (Sl. No. 8-12, and 20-24), and some formaldehyde based copolymer resins (Sl. No. 3, 4, 15 (a & b) and 18) were effective against both Gram-positive and Gram-negative bacteria, with a degree of variation in their activity. 2,4-DHPPE formaldehyde-orthohydroxybenzoic acid copolymer resin (Sl. No. 16) was selective in its action as it permitted the growth of *B. subtilis* and *St. aureus* but failed to inhibit the growth of both the Gram-negative bacteria *(E. coli* and *Serretia* sp.).

All the four (2,4-DHPP-FM; 2,4-DHPP-FF; 2,4-DHPPE-FM and 2,4-DHPPEFF) homo resins were inactive against all the four strains of bacteria, employed in the investigation. 2,4-DHPPE-formaldehyde-paraaminobenzoic acid (Sl. No. 18) exhibited a poor to moderate antibacterial activity hence it is less effective as antibacterial agent. 2,4-DHPP-formaldehyde-parachlorobenzoic acid (Sl. No. 3), 2,4-DHPPE-formaldehyde-parachlorobenzoic acid (Sl. No. 15) and 2,4-DHPPE-furfural parachlorobenzoic acid (Sl.No.21) copolymer resins were more potent antibacterial agents surpassing even the streptomycin sulphate standard employed. The next to these, 2,4-DHPP-furfural-paraaminobenzoic acid (Sl.No. 12), 2,4-DHPPE-furfural-orthohydroxybenzoic acid (Sl. No. 22), 2,4-DHPPE-furfural-paraaminobenzoic acid (Sl. No. 24) copolymer resins, were equipotent with that of the antibiotic streptomycin used as standard.

In general resins derived from parachlorobenzoic acid (comonomer) were more potent antibacterials. Next being para-aminobenzoic acid-furfural based resin copolymers which were more toxic than formaldehyde based resins. Similarly copolymer resins prepared using acid catalyst were more toxic than those prepared from base catalyst (Sl.No. 15a & 15b). Gram-negative bacteria were comparatively more sensitive than Gram positive bacteria.

Many parameters may influence the antimicrobial activity; modification of the biocidal group during interaction with bacteria, acidity or alkalinity of the medium, hardness of water, uv radiation, adsorption of organic products etc. Antibacteiral activity of the resins under study could be referred to a number of causes like injurious effect on the cell wall or cell division, effect on permeability of cell membrane and cell enzyme system, chelation and precipitation of chemicals. Oxygen and nitrogen atoms present in the resin can act as hydrogen acceptor in the metabolic system and by doing so disturb the normal hydrogenation and dehydrogenation reactions in the cell. This might be the reason for high activity of furfural based resins. The high activity of parachlorobenzoic acid based resin copolymers against all pathogenic bacteria may be due to the presence of chlorine atom at the para position, free from steric hindrance.

The biological activity of hydroquinone is well established[21]. The relative reduced bacterial sensitivity of orthosubstituted benzoic acid based resin copolymers may be attributed to the lower composition of hydro quinone unit in the copolymer. In case of other copolymer resins a synergistic structural effects may be playing role in antibacterial activity.

The present observations may serve as a guide for designing new antibacterial materials that have the advantage of being non-polluting. Ideally, the activity may be. permanent because the biocidal group (comonomer) is not consumed during the course of interaction with microorganisms.

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